(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 4 October 2001 (04.10.2001)

PCT

(10) International Publication Number WO 01/72774 A2

(51) International Patent Classification⁷: C07K 14/00

(21) International Application Number: PCT/GB01/01297

(22) International Filing Date: 23 March 2001 (23.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0007268.6 24 March 2000 (24.03.2000) GH

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

 without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



(54) Title: CELL CYCLE PROGRESSION PROTEINS

(57) Abstract: Polynucleotides encoding a number of *Drosophila* gene products are provided. Polynucleotide probes derived from these nucleotide sequences, polypeptides encoded by the polynucleotides and antibodies that bind to the polypeptides are also provided.

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CELL CYCLE PROGRESSION PROTEINS

The present invention relates to a number of genes implicated in the processes of cell cycle progression, including mitosis and meiosis.

We have now identified a large number of genes in *Drosophila*, mutations in which disrupt cell cycle progression, for example the processes of mitosis and/or meiosis. We have determined the phenotypes of these mutations and recovered nucleotide sequences associated with the corresponding genes. Many of these nucleotide sequences correspond to protein open reading frames (ORFs) present in the *Drosophila* genome.

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Accordingly the present invention provides in one aspect a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 1 to 70 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 1 to 70, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 1 to 70 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

There is provided, according to another aspect of the present invention, a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 1 to 14 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 1 to 14, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 1 to 14 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

We provide, according to yet a further aspect of the present invention, a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide

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sequences set out in Examples 15 to 19 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 15 to 19, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 15 to 19 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

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As a further aspect of the present invention, there is provided a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 20 to 30 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 20 to 30, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 20 to 30 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

We provide, according to a yet further aspect of the present invention, a polynucleotide selected from: (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 31 to 53 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 31 to 53, or a fragment thereof; (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in 31 to 53 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

The present invention, in a further aspect, provides a polynucleotide selected from:
(a) polynucleotides comprising any one of the nucleotide sequences set out in 54 to 70 or the complement thereof; (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in 54 to 70, or a fragment thereof; (c)

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polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in 54 to 70 or a fragment thereof; (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

A polynucleotide probe which comprises a fragment of at least 15 nucleotides of a polynucleotide according to any of the above aspects of the invention.

The present invention also provides a polypeptide which comprises any one of the amino acid sequences set out in Examples 1 to 70 or in any of Examples 1 to 14, Examples 15 to 19, Examples 20 to 30, Examples 31 to 53 and Examples 54 to 70, or a homologue, variant, derivative or fragment thereof.

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Preferably the polypeptide is encoded by a cDNA sequence obtainable from a eukaryotic cDNA library, preferably a metazoan cDNA library (such as insect or mammalian) said DNA sequence comprising a DNA sequence being selectively detectable with a *Drosophila* nucleotide sequence as shown in any one of Examples 1 to 70.

The term "selectively detectable" means that the cDNA used as a probe is used under conditions where a target cDNA of the invention is found to hybridize to the probe at a level significantly above background. The background hybridization may occur because of other cDNAs present in the cDNA library. In this event background implies a level of signal generated by interaction between the probe and a non-specific cDNA member of the library which is less than 10 fold, preferably less than 100 fold as intense as the specific interaction observed with the target cDNA. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ³²P. Suitable conditions may be found by reference to the Examples, as well as in the detailed description below.

A polynucleotide encoding a polypeptide of the invention is also provided.

The present invention further provides a vector comprising a polynucleotide of the invention, for example an expression vector comprising a polynucleotide of the invention

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operably linked to a regulatory sequence capable of directing expression of said polynucleotide in a host cell.

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Also provided is an antibody capable of binding a polypeptide of the invention.

In a further aspect the present invention provides a method for detecting the presence or absence of a polynucleotide of the invention in a biological sample which method comprises: (a) bringing the biological sample containing DNA or RNA into contact with a probe comprising a nucleotide of the invention under hybridising conditions; and (b) detecting any duplex formed between the probe and nucleic acid in the sample.

In another aspect the invention provides a method for detecting a polypeptide of the invention present in a biological sample which comprises: (a) providing an antibody of the invention; (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said antibody is formed.

Knowledge of the genes involved in cell cycle progression allows the development of therapeutic agents for the treatment of medical conditions associated with aberrant cell cycle progression. Accordingly, the present invention provides a polynucleotide of the invention for use in therapy. The present invention also provides a polypeptide of the invention for use in therapy. The present invention further provides an antibody of the invention for use in therapy.

In a specific embodiment, the present invention provides a method of treating a tumour or a patient suffering from a proliferative disease, comprising administering to a patient in need of treatment an effective amount of a polynucleotide, polypeptide and/or antibody of the invention.

The present invention also provides the use of a polypeptide of the invention in a method of identifying a substance capable of affecting the function of the corresponding

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gene. For example, in one embodiment the present invention provides the use of a polypeptide of the invention in an assay for identifying a substance capable of inhibiting cell cycle progression. The substance may inhibit any of the steps or stages in the cell cycle, for example, formation of the nuclear envelope, exit from the quiescent phase of the cell cycle (G0), G1 progression, chromosome decondensation, nuclear envelope breakdown, START, initiation of DNA replication, progression of DNA replication, termination of DNA replication, centrosome duplication, G2 progression, activation of mitotic or meiotic functions, chromosome condensation, centrosome separation, microtubule nucleation, spindle formation and function, interactions with microtubule motor proteins, chromatid separation and segregation, inactivation of mitotic functions, formation of contractile ring, and cytokinesis functions. For example, possible functions of genes of the invention for which it may be desired to identify substances which affect such functions include chromatin binding, formation of replication complexes, replication licensing, phosphorylation or other secondary modification activity, proteolytic degradation, microtubule binding, actin binding, septin binding, microtubule organising centre nucleation activity and binding to components of cell cycle signalling pathways.

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In a further aspect the present invention provides a method for identifying a substance capable of binding to a polypeptide of the invention, which method comprises incubating the polypeptide with a candidate substance under suitable conditions and determining whether the substance binds to the polypeptide.

In an additional aspect, the invention provides kits comprising polynucleotides, polypeptides or antibodies of the invention and methods of using such kits in diagnosing the presence of absence of polynucleotides and polypeptides of the invention including deleterious mutant forms.

Also provided is a substance identified by the above methods of the invention. Such substances may be used in a method of therapy, such as in a method of affecting cell cycle progression, for example mitosis and/or meiosis.

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The invention also provides a process comprising the steps of: (a) performing one of the above methods; and (b) preparing a quantity of those one or more substances identified as being capable of binding to a polypeptide of the invention.

Also provided is a process comprising the steps of: (a) performing one of the above methods; and (b) preparing a pharmaceutical composition comprising one or more substances identified as being capable of binding to a polypeptide of the invention.

We further provide a method for identifying a substance capable of modulating the function of a polypeptide of the invention or a polypeptide encoded by a polynucleotide of the invention, the method comprising the steps of: incubating the polypeptide with a candidate substance and determining whether activity of the polypeptide is thereby modulated.

A substance identified by a method or assay according to any of the above methods or processes is also provided, as is the use of such a substance in a method of inhibiting the function of a polypeptide. Use of such a substance in a method of regulating a cell division cycle function is also provided.

DETAILED DESCRIPTION OF THE INVENTION

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The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; and, D.

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M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A:* Synthesis and Physical Analysis of DNA Methods in Enzymology, Academic Press. Each of these general texts is herein incorporated by reference.

Preferably, the polypeptides and polynucleotides of the invention are such that they give rise to or are associated with defined phenotypes when mutated.

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For example, mutations in the polypeptides and polynucleotides of the invention may be associated with a failure to complete cytokinesis; such polypeptides and polynucleotides are conveniently categorised as "Category 1". Phenotypes associated with Category 1 polypeptides and polynucleotides include any one or more of the following, singly or in combination: Mitotic defects in brain: cytokinesis defect (polyploidy); Male semi-sterile, Meiotic defects in testis: cytokinesis defects, segregation defects.(Seg-01/62); Meiotic defects in testis: cytokinesis defects, abnormal spindles.(Ab-02/12); Mitotic defects in brain: cytokinesis defect (no overcondensation of diploids, high polyploidy); Meiotic defects in testis: cytokinesis defects. Dark bands in eyes, dominant; Meiotic defects in testis: cytokinesis defects; Meiotic defects in testis:segregation defect, cytokinesis defect(Ck-09/32); Mitotic defects in brain: cytokinesis defect (no overcondensation of diploids, very high polyploidy); Mitotic defects in brain: cytokinesis defect(very high polyploidy); Mitotic defects in brain: cytokinesis defect. Meiotic defects in testis: cytokinesis defects (Mitotic higher level of condensation, polyploidy, Meiotic: Ck05/07); Mitotic defects in brain, Cytokinesis defect (no overcondensation of diploids, high polyploidy); Mitotic defects in brain: cytokinesis defect (very high polyploidy, chromosomes entangled?); Mitotic defects in brain: cytokinesis defect (very high polyploidy: Meiotic defects in testis: cytokinesis defects (Ck-04/06) '; Female sterile (anaphase bridges, lagging chromosomes); Mitotic defects in brain: cytokinesis defect. Meiotic defects in testis: cytokinesis defects:(mitotic: high polyploidy, no diploids, higher mitotic index, meiotic: Ck-01/05); Meiotic defects in testis: cytokinesis defects; Meiotic defects in testis: cytokinesis defects(Ck-06/09); Meiotic defects in testis: segregation defects, cytokinesis defect(Ck-07/35); Meiotic defects in testis: cytokinesis defects.

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Alternatively, mutations in the polypeptides and polynucleotides of the invention may be associated with a failure to enter M-phase; such polypeptides and polynucleotides are conveniently categorised as "Category 2". Phenotypes associated with Category 2 polypeptides and polynucleotides include any one or more of the following, singly or in combination: Meiotic defects in testis: no division(no meiosis); Mitotic defects in brain: no mitosis; Meiotic defects in testis: segregation defects, meiotic failure(Mf-07/75); Meiotic defects in testis: segregation defects, meiotic failure(Mf-05/31); Meiotic defects in testis: cytokinesis defects, meiotic failure(Mf-02/15).

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Mutations in the polypeptides and polynucleotides of the invention may be associated with a metaphase arrest phenotype ("Category 3"). Phenotypes associated with Category 3 polypeptides and polynucleotides include any one or more of the following, singly or in combination: Mitotic defects in brain: prometaphase arrest (overcondensation, polyploidy, scattered chromosomes with bipolar spindle); Male sterile, Female sterile, Mitotic defects in brain: prometaphase arrest (Overcondensation, polyploidy, fewer anaphases, high mitotic index, scattered chromosomes with bipolar spindle); Mitotic defects in brain: (weak overcondensation, metaphase with bipolar spindle); Mitotic defects in brain: prometaphase arrest; Mitotic defects in brain: metaphase arrest; Mitotic defects in brain: metaphase arrest. (overcondensation, polyploidy, aneuploidy, few anaphases, high mitotic index, metaphase with bent bipolar spindle); Mitotic defects in brain: metaphase arrest. (overcondensation, polyploidy, few anaphases, high mitotic index, metaphase with bent bipolar spindle); Mitotic defects in brain: Metaphase arrest (overcondensation, polyploidy, aneuploidy, no anaphases, high mitotic index, metaphase with bipolar spindle); Mitotic defects in brain: metaphase arrest (overcondensation, metaphase with bipolar spindle; Meiotic defects in testis: segregation defects, multipolar spindles (Mul-02/29); Meiotic defects in testis: cytokinesis defects, abnormal spindles. (Ab-01/03); Mitotic defects in brain; metaphase arrest; Mitotic defects in brain: metaphase arrest (overcondensation, polyploidy, metaphase with bipolar spindle); Mitotic defects in brain: metaphase arrest. Meiotic defects in testis: segregation defects. Abnormal spindles (mitotic: High mitotic index, meiotic: Ab-08/24); Mitotic defects in brain: metaphase arrest(overcondensation, few anaphases, some polyploids); Mitotic defects in brain: prometaphase arrest (overcondensation, fewer anaphases, metaphase with bipolar spindle):

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Mitotic defects in brain: metaphase arrest(condensation, no polyploidy, no anaphases, metaphase with bipolar spindle).

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Mutations in Category 4 polypeptides and polynucleotides of the invention may be associated with an anaphase defect phenotype; phenotypes associated with Category 4 polypeptides and polynucleotides include any one or more of the following, singly or in combination: Mitotic defects in brain: anaphase defects (overcondensation, high polyploidy, some lagging chromosomes); Meiotic defects in testis: segregation defects; Male and female sterile, small wings, meiotic defects in testis: segregation defects, elongation defect; Mitotic defects in brain: anaphase defects(overcondensation, anaphase bridge, metaphase with swollen chromosomes and bipolar spindle); Mitotic defects in brain: Anaphase defects. (overcondensation, aneuploidy, some lagging chromosomes and breaks); Meiotic defects in testis: segregation defects; Meiotic defects in testis: segregation defects, multi-stage defects (Pl-02/17); Meiotic defects in testis: segregation defects, multi-stage defects (Pl-02/18); Meiotic defects in testis: cytokinesis defects, segregation defects (seg-01/01); Mitotic defects in brain: cytokinesis defect. Meiotic defects in testis: cytokinesis defect. Multi-stage defects Polyploidy, no overcondensation Pl-01/10; Meiotic defects in testis: segregation defects, abnormal spindles. (Ab-03/30); Mitotic defects in brain: anaphase defects (weak, higher condensation, some polyploidy, fewer anaphases, polyploids with monopolar spindles); Mitotic defects in brain: anaphase defects (overcondensation, polyplody (with overcondensation), few anaphases, metaphase with bipolar spindle); Meiotic defects in testis: cytokinesis defects; Meiotic defects in testis: segregation defects, multipolar spindles (Mul-02/22); Meiotic defects in testis: segregation defects, abnormal spindles (Ab-04/26); Meiotic defects in testis: cytokinesis defects, abnormal spindles (Ab-16/13); Mitotic defects in brain: anaphase defects. Meiotic defects in testis: segregation defects, abnormal spindles (mitotic: Overcondensation, lagging chromosomes/less aligned metaphase with bipolar spindles, Meiotic: Ab-06/20); Meiotic defects in testis: segregation defects; Meiotic defects in testis: no division (no meiosis); Meiotic defects in testis: segregation defects, abnormal spindles (Ab-12/48); Meiotic defects in testis: segregation defects, multipolar spindles(mitotic: High polyploids, no diploids, higher mitotic index Meiotic: Mul-02/59); Meiotic defects in testis: segregation defect; Meiotic defects in testis: segregation defects, abnormal spindles

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(meiotic: Ab-08/42); Female sterile. Meiotic defects in testis: cytokinesis defects, segregation defects (Mitotic: Less condensed chromosomes, nuclear bridges, Meiotic: Seg-01/02; Mitotic defects in brain: anaphase defects; Meiotic defects in testis: cytokinesis defects, abnormal spindles(Ab-01/04); Meiotic defects in testis: segregation defects(overcondensation, fewer anaphases); Mitotic defects in brain:(some overcondensation, anaphase bridge, metaphase with swollen chromosome and bipolar spindle).

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A fifth category ("Category 5") of polypeptides and polynucleotides of the invention are associated with the presence of small imaginal discs (block to proliferation). Phenotypes associated with Category 5 polypeptides and polynucleotides include any one or more of the following, singly or in combination: 2nd chromosome, small imaginal discs.

The polypeptides and polynucleotides of the invention may also be categorised according to their function, or their putative function.

For example, the polypeptides described here preferably comprise, and the polynucleotides described here are ones which preferably encode polypeptides comprising, any one or more of the following: a CBP activator protein; a CCR4-associated regulator of polymerase II transcription; a CTP synthase (CTPS); a Cyclin specific ubiquitin conjugating enzyme; a DNA packaging protein; a DNA repair protein; a DNA-binding protein involved in chromosomal organisation; a DNase IV; a EIF4G2 translation initiation factor; a eukaryotic translation initiation factor 6; a Ecdysone-induced protein 78C; a Egf2 translation factor; a G protein-coupled receptor kinase 7; a GTPase exchange factor; a phosphatidylinositol transfer protein beta isoform; a His-rich protein; a Lk6 kinase; a MAP kinase; a MAP kinase interacting kinase 1; a N-arginine dibasic convertase; a Phosphatidylinositol transfer protein; a RIP protein kinase; a RNA binding motif, single stranded interacting protein; a RNA binding protein; a RYKreceptor tyrosine kinase; a Ribosomal protein L1; a selenide, water dikinase 1; a selenium donor protein 1; a selenophosphate synthetase 1; a Sqv-7-like protein; a sugar modification protein; a protein involved in cytokinesis and signalling; a TEK tyrosine kinase; a Translation elongation

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factor; a UDP-galactose transporter; a v-erba related protein; a WD40 protein; a brahma protein; a calcium binding protein; a cell adhesion protein; a chaperone; a chromodomain helicase DNA binding protein; a chromodomain-helicase-DNA-binding protein; a coiled coil protein with ubiquitin like domain; a component of the 19S proteasome regulatory particle; a couch potato RNA binding protein; a cytidine 5-prime triphosphate synthetasea; a cytoskeletal structural protein; a death domain containing protein; a developmentally expressed in axons of the CNS; a diacylglycerol-activated/phosholipid dependent protein kinase C inhibitor; a diazepam binding inhibitor; a diphosphate kinase; a dodecasattelite DNA binding protein; a doughnut protein tyrosine kinase; an elongation factor 2; a endoplasmic reticulum ATPase; a eukaryotic translation initiation factor 4E binding protein 2; a factor involved in axon guidance; a fatty-acid-Coenzyme A ligase; a flap structure-specific endonuclease 1; a protein involved in the formation of the contractile ring and the initiation of cytokinesis; a glucose-6-phosphate transporter; a glycoprotein glucosyltransferase; a growth factor; a transmembrane receptor protein tyrosine kinase involved in cell growth and maintenance; a guanyl-nucleotide exchange factor involved in signal transduction; a heat shock protein; a helicase; a high density lipoprotein binding protein; a histone acetyl transferase transcriptional activator; a histone acetyltransferase; a histone acetyltransferase GCN5; a protein involved in development of the abdomen (embryos); a protein involved in the development of the imaginal discs (larvae or pupae); a kinesin like protein 67a; a ligand-dependent nuclear receptor; a ligand-dependent nuclear receptor; a lola-like specific RNA polymerase II transcription factor; a matrix associated protein; a membrane glycoprotein; a mitotic heterochromatin fragment clone CH(2)6; a motor protein; a motor protein involved in cytoskeleton organization; a mushroom body RNA binding protein; a myosin like proteins; a nemo-like kinase; a non-ATPase protein; a nuclear receptor NR1E1; a nucleic acid binding protein; a nucleoside diphosphate kinase (NBR-A); a oly(rC)-binding protein 2 (hnRNP-E1); a peroxisome biogenesis factor 1; a phosopholipid transporter involved in lipid metabolism; a phosphatase or enhancer of Pi uptake protein; a protease; a proteasome regulatory particle; a protein involved in cytoskeleton organization and/or biogenesis; a protein kinase associated with microtubules; a protein kinase mitogen-activated 7; a protein serine/threonine kinase involved in cell cycle, possibly targeted to cytoskeleton; a protein serine/threonine kinase involved in eye morphogenesis; a protein which associates with cdc25 phosphatase; a

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protein which induces apoptosis; a ribonuclease P; a ribonuclease P protein subunit p29; a ser/thr phosphatase; a signal transduction protein; a signal transport protein; a sin3associated polypeptide; a single stranded DNA/RNA binding protein; a sodium-dependent dicarboxylate transporters; a ssDNA/RNA binding proteins; a striatin, calmodulin-binding protein (STRN); a structural protein of ribosome involved in protein biosynthesis; a subtelomeric heterochromatin repeats; a sugar acetylase; a sugar modification protein; a suppresspr of ras; a tRNA processing enzyme Ribonuclease P protein subunit; a thyroid hormone responsive gene; a tie receptor protein tyrosine kinase; a transacylase; a transcription factor; a transcription factor involved in chromatin remodelling; a transcriptional regulation of c-myc expression; a transcriptional regulator; a transcriptional regulators/telomeric silencing; a translation initiation factor; a tumor metastasis inhibitor; a tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein; a ubiquitin carrier protein; a ubiquitin-conjugating enzyme; a ugtUDP-glucose-glycoprotein glucosyltransferase; a zinc finger protein; an RNA polymerase II transcription factor; an acetylcholinesterase (YT blood group) precursor; an actin binding protein; an actin dependent regulator of chromatin; an acyl-CoA-binding protein; an alanine:glyoxylate aminotransferase; an alpha esterase; an ankyrin protein; an imitation-SWI protein; and an integrin beta 4 binding protein.

POLYPEPTIDES

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It will be understood that polypeptides of the invention are not limited to polypeptides having the amino acid sequence set out in Examples 1 to 70 or fragments thereof but also include homologous sequences obtained from any source, for example related viral/bacterial proteins, cellular homologues and synthetic peptides, as well as variants or derivatives thereof.

Thus polypeptides of the invention also include those encoding homologues from other species including animals such as mammals (e.g. mice, rats or rabbits), especially primates, more especially humans. More specifically, homologues included within the scope of the invention include human homologues.

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Thus, the present invention covers variants, homologues or derivatives of the amino acid sequence set out in Examples 1 to 70, as well as variants, homologues or derivatives of the nucleotide sequence coding for the amino acid sequences of the present invention.

In the context of the present invention, a homologous sequence is taken to include an amino acid sequence which is at least 15, 20, 25, 30, 40, 50, 60, 70, 80 or 90% identical, preferably at least 95 or 98% identical at the amino acid level over at least 50 or 100, preferably 200, 300, 400 or 500 amino acids with any one of the polypeptide sequences shown in the Examples. In particular, homology should typically be considered with respect to those regions of the sequence known to be essential for protein function rather than non-essential neighbouring sequences. This is especially important when considering homologous sequences from distantly related organisms.

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Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These publicly and commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues (for example less than 50 contiguous amino acids).

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus

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potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

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However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A; Devereux *et al.*, 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.*, 1999 *ibid* – Chapter 18), FASTA (Atschul *et al.*, 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.*, 1999 *ibid*, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program.

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each

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pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

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Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The terms "variant" or "derivative" in relation to the amino acid sequences of the present invention includes any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acids from or to the sequence providing the resultant amino acid sequence retains substantially the same activity as the unmodified sequence, preferably having at least the same activity as the polypeptides presented in the sequence listings in the Examples.

Polypeptides having the amino acid sequence shown in the Examples, or fragments or homologues thereof may be modified for use in the present invention. Typically, modifications are made that maintain the biological activity of the sequence. Amino acid substitutions may be made, for example from 1, 2 or 3 to 10, 20 or 30 substitutions provided that the modified sequence retains the biological activity of the unmodified sequence. Alternatively, modifications may be made to deliberately inactivate one or more functional domains of the polypeptides of the invention. Amino acid substitutions may include the use of non-naturally occurring analogues, for example to increase blood plasma half-life of a therapeutically administered polypeptide.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

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ALIPHATIC	Non-polar	GAP
		ILV
	Polar - uncharged	CSTM
		NQ
	Polar - charged	DE
		KR
AROMATIC		HFWY

Polypeptides of the invention also include fragments of the full length sequences mentioned above. Preferably said fragments comprise at least one epitope. Methods of identifying epitopes are well known in the art. Fragments will typically comprise at least 6 amino acids, more preferably at least 10, 20, 30, 50 or 100 amino acids.

Proteins of the invention are typically made by recombinant means, for example as described below. However they may also be made by synthetic means using techniques well known to skilled persons such as solid phase synthesis. Proteins of the invention may also be produced as fusion proteins, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis,

GAL4 (DNA binding and/or transcriptional activation domains) and β-galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. Preferably the fusion protein will not hinder the function of the protein of interest sequence. Proteins of the invention may also be obtained by purification of cell extracts from animal cells.

Proteins of the invention may be in a substantially isolated form. It will be understood that the protein may be mixed with carriers or diluents which will not interfere with the intended purpose of the protein and still be regarded as substantially isolated. A protein of the invention may also be in a substantially purified form, in which case it will generally comprise the protein in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the protein in the preparation is a protein of the invention.

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A polypeptide of the invention may be labeled with a revealing label. The revealing label may be any suitable label which allows the polypeptide to be detected. Suitable labels include radioisotopes, e.g. ¹²⁵I, enzymes, antibodies, polynucleotides and linkers such as biotin. Labeled polypeptides of the invention may be used in diagnostic procedures such as immunoassays to determine the amount of a polypeptide of the invention in a sample. Polypeptides or labeled polypeptides of the invention may also be used in serological or cell-mediated immune assays for the detection of immune reactivity to said polypeptides in animals and humans using standard protocols.

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A polypeptide or labeled polypeptide of the invention or fragment thereof may also be fixed to a solid phase, for example the surface of an immunoassay well or dipstick. Such labeled and/or immobilised polypeptides may be packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like. Such polypeptides and kits may be used in methods of detection of antibodies to the polypeptides or their allelic or species variants by immunoassay.

Immunoassay methods are well known in the art and will generally comprise: (a) providing a polypeptide comprising an epitope bindable by an antibody against said protein; (b) incubating a biological sample with said polypeptide under conditions which allow for the formation of an antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said polypeptide is formed.

Polypeptides of the invention may be used in *in vitro* or *in vivo* cell culture systems to study the role of their corresponding genes and homologues thereof in cell function, including their function in disease. For example, truncated or modified polypeptides may be introduced into a cell to disrupt the normal functions which occur in the cell. The polypeptides of the invention may be introduced into the cell by *in situ* expression of the polypeptide from a recombinant expression vector (see below). The expression vector optionally carries an inducible promoter to control the expression of the polypeptide.

The use of appropriate host cells, such as insect cells or mammalian cells, is expected to provide for such post-translational modifications (e.g. myristolation,

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glycosylation, truncation, lapidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention. Such cell culture systems in which polypeptides of the invention are expressed may be used in assay systems to identify candidate substances which interfere with or enhance the functions of the polypeptides of the invention in the cell.

POLYNUCLEOTIDES

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Polynucleotides of the invention include polynucleotides that comprise any one or more of the nucleic acid sequences set out in Examples 1 to 70 and fragments thereof. Polynucleotides of the invention also include polynucleotides encoding the polypeptides of the invention. It will be understood by a skilled person that numerous different polynucleotides can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides of the invention to reflect the codon usage of any particular host organism in which the polypeptides of the invention are to be expressed.

Polynucleotides of the invention may comprise DNA or RNA. They may be single-stranded or double-stranded. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present invention, it is to be understood that the polynucleotides described herein may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of polynucleotides of the invention.

The terms "variant", "homologue" or "derivative" in relation to the nucleotide sequence of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the

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sequence. Preferably said variant, homologues or derivatives code for a polypeptide having biological activity.

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As indicated above, with respect to sequence homology, preferably there is at least 50 or 75%, more preferably at least 85%, more preferably at least 90% homology to the sequences shown in the sequence listing herein. More preferably there is at least 95%, more preferably at least 98%, homology. Nucleotide homology comparisons may be conducted as described above. A preferred sequence comparison program is the GCG Wisconsin Bestfit program described above. The default scoring matrix has a match value of 10 for each identical nucleotide and -9 for each mismatch. The default gap creation penalty is -50 and the default gap extension penalty is -3 for each nucleotide.

The present invention also encompasses nucleotide sequences that are capable of hybridising selectively to the sequences presented herein, or any variant, fragment or derivative thereof, or to the complement of any of the above. Nucleotide sequences are preferably at least 15 nucleotides in length, more preferably at least 20, 30, 40 or 50 nucleotides in length.

The term "hybridization" as used herein shall include "the process by which a strand of nucleic acid joins with a complementary strand through base pairing" as well as the process of amplification as carried out in polymerase chain reaction technologies.

Polynucleotides of the invention capable of selectively hybridising to the nucleotide sequences presented herein, or to their complement, will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% or 98% homologous to the corresponding nucleotide sequences presented herein over a region of at least 20, preferably at least 25 or 30, for instance at least 40, 60 or 100 or more contiguous nucleotides.

The term "selectively hybridizable" means that the polynucleotide used as a probe is used under conditions where a target polynucleotide of the invention is found to hybridize to the probe at a level significantly above background. The background

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hybridization may occur because of other polynucleotides present, for example, in the cDNA or genomic DNA library being screening. In this event, background implies a level of signal generated by interaction between the probe and a non-specific DNA member of the library which is less than 10 fold, preferably less than 100 fold as intense as the specific interaction observed with the target DNA. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ³²P.

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Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA), and confer a defined "stringency" as explained below.

Maximum stringency typically occurs at about Tm-5°C (5°C below the Tm of the probe); high stringency at about 5°C to 10°C below Tm; intermediate stringency at about 10°C to 20°C below Tm; and low stringency at about 20°C to 25°C below Tm. As will be understood by those of skill in the art, a maximum stringency hybridization can be used to identify or detect identical polynucleotide sequences while an intermediate (or low) stringency hybridization can be used to identify or detect similar or related polynucleotide sequences.

In a preferred aspect, the present invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under stringent conditions (e.g. 65° C and 0.1xSSC {1xSSC = 0.15 M NaCl, 0.015 M Na₃ Citrate pH 7.0).

Where the polynucleotide of the invention is double-stranded, both strands of the duplex, either individually or in combination, are encompassed by the present invention. Where the polynucleotide is single-stranded, it is to be understood that the complementary sequence of that polynucleotide is also included within the scope of the present invention.

Polynucleotides which are not 100% homologous to the sequences of the present invention but fall within the scope of the invention can be obtained in a number of ways. Other variants of the sequences described herein may be obtained for example by probing

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DNA libraries made from a range of individuals, for example individuals from different populations. In addition, other viral/bacterial, or cellular homologues particularly cellular homologues found in mammalian cells (e.g. rat, mouse, bovine and primate cells), may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the Examples. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal species, and probing such libraries with probes comprising all or part of any on of the sequences shown in the Examples under conditions of medium to high stringency. The nucleotide sequences of the human homologues described in the Examples, may preferably be used to identify other primate/mammalian homologues since nucleotide homology between human sequences and mammalian sequences is likely to be higher than is the case for the *Drosophila* sequences identified herein.

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Similar considerations apply to obtaining species homologues and allelic variants of the polypeptide or nucleotide sequences of the invention.

Variants and strain/species homologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the variants and homologues encoding conserved amino acid sequences within the sequences of the present invention. Conserved sequences can be predicted, for example, by aligning the amino acid sequences from several variants/homologues. Sequence alignments can be performed using computer software known in the art. For example the GCG Wisconsin PileUp program is widely used.

The primers used in degenerate PCR will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences. It will be appreciated by the skilled person that overall nucleotide homology between sequences from distantly related organisms is likely to be very low and thus in these situations degenerate PCR may be the method of choice rather than screening libraries with labeled fragments the sequences disclosed in the Examples.

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In addition, homologous sequences may be identified by searching nucleotide and/or protein databases using search algorithms such as the BLAST suite of programs. This approach is described in the Examples.

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Alternatively, such polynucleotides may be obtained by site directed mutagenesis of characterised sequences, such as the sequences disclosed in the Examples. This may be useful where for example silent codon changes are required to sequences to optimise codon preferences for a particular host cell in which the polynucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the property or function of the polypeptides encoded by the polynucleotides. For example, further changes may be desirable to represent particular coding changes found in the sequences disclosed in the Examples which give rise to mutant genes which have lost their regulatory function. Probes based on such changes can be used as diagnostic probes to detect such mutants.

Polynucleotides of the invention may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labeled with a revealing label by conventional means using radioactive or non-radioactive labels, or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 8, 9, 10, or 15, preferably at least 20, for example at least 25, 30 or 40 nucleotides in length, and are also encompassed by the term polynucleotides of the invention as used herein.

Polynucleotides such as a DNA polynucleotides and probes according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

In general, primers will be produced by synthetic means, involving a step wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

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Longer polynucleotides will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking a region of the lipid targeting sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector

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Polynucleotides or primers of the invention may carry a revealing label. Suitable labels include radioisotopes such as ³²P or ³⁵S, enzyme labels, or other protein labels such as biotin. Such labels may be added to polynucleotides or primers of the invention and may be detected using by techniques known *per se*.

Polynucleotides or primers of the invention or fragments thereof labeled or unlabeled may be used by a person skilled in the art in nucleic acid-based tests for detecting or sequencing polynucleotides of the invention in the human or animal body.

Such tests for detecting generally comprise bringing a biological sample containing DNA or RNA into contact with a probe comprising a polynucleotide or primer of the invention under hybridising conditions and detecting any duplex formed between the probe and nucleic acid in the sample. Such detection may be achieved using techniques such as PCR or by immobilising the probe on a solid support, removing nucleic acid in the sample which is not hybridised to the probe, and then detecting nucleic acid which has hybridised to the probe. Alternatively, the sample nucleic acid may be immobilised on a solid support, and the amount of probe bound to such a support can be detected. Suitable assay methods of this and other formats can be found in for example WO89/03891 and WO90/13667.

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Tests for sequencing nucleotides of the invention include bringing a biological sample containing target DNA or RNA into contact with a probe comprising a polynucleotide or primer of the invention under hybridising conditions and determining the sequence by, for example the Sanger dideoxy chain termination method (see Sambrook *et al.*).

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Such a method generally comprises elongating, in the presence of suitable reagents, the primer by synthesis of a strand complementary to the target DNA or RNA and selectively terminating the elongation reaction at one or more of an A, C, G or T/U residue; allowing strand elongation and termination reaction to occur; separating out according to size the elongated products to determine the sequence of the nucleotides at which selective termination has occurred. Suitable reagents include a DNA polymerase enzyme, the deoxynucleotides dATP, dCTP, dGTP and dTTP, a buffer and ATP. Dideoxynucleotides are used for selective termination.

Tests for detecting or sequencing nucleotides of the invention in a biological sample may be used to determine particular sequences within cells in individuals who have, or are suspected to have, an altered gene sequence, for example within cancer cells including leukaemia cells and solid tumours such as breast, ovary, lung, colon, pancreas, testes, liver, brain, muscle and bone tumours. Cells from patients suffering from a proliferative disease may also be tested in the same way.

In addition, the identification of the genes described in the Examples will allow the role of these genes in hereditary diseases to be investigated. In general, this will involve establishing the status of the gene (e.g. using PCR sequence analysis), in cells derived from animals or humans with, for example, neurological disorders or neoplasms.

The probes of the invention may conveniently be packaged in the form of a test kit
in a suitable container. In such kits the probe may be bound to a solid support where the
assay format for which the kit is designed requires such binding. The kit may also contain
suitable reagents for treating the sample to be probed, hybridising the probe to nucleic acid
in the sample, control reagents, instructions, and the like.

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NUCLEIC ACID VECTORS

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Polynucleotides of the invention can be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Thus in a further embodiment, the invention provides a method of making polynucleotides of the invention by introducing a polynucleotide of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells include bacteria such as *E. coli*, yeast, mammalian cell lines and other eukaryotic cell lines, for example insect Sf9 cells.

Preferably, a polynucleotide of the invention in a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" means that the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under condition compatible with the control sequences.

The control sequences may be modified, for example by the addition of further transcriptional regulatory elements to make the level of transcription directed by the control sequences more responsive to transcriptional modulators.

Vectors of the invention may be transformed or transfected into a suitable host cell as described below to provide for expression of a protein of the invention. This process may comprise culturing a host cell transformed with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the protein, and optionally recovering the expressed protein. Vectors will be chosen that are compatible with the host cell used.

The vectors may be for example, plasmid or virus vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and

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optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector. Vectors may be used, for example, to transfect or transform a host cell.

Control sequences operably linked to sequences encoding the polypeptide of the invention include promoters/enhancers and other expression regulation signals. These control sequences may be selected to be compatible with the host cell for which the expression vector is designed to be used in. The term promoter is well-known in the art and encompasses nucleic acid regions ranging in size and complexity from minimal promoters to promoters including upstream elements and enhancers.

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The promoter is typically selected from promoters which are functional in mammalian cells, although prokaryotic promoters and promoters functional in other eukaryotic cells, such as insect cells, may be used. The promoter is typically derived from promoter sequences of viral or eukaryotic genes. For example, it may be a promoter derived from the genome of a cell in which expression is to occur. With respect to eukaryotic promoters, they may be promoters that function in a ubiquitous manner (such as promoters of α -actin, β -actin, tubulin) or, alternatively, a tissue-specific manner (such as promoters of the genes for pyruvate kinase). They may also be promoters that respond to specific stimuli, for example promoters that bind steroid hormone receptors. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR) promoter, the rous sarcoma virus (RSV) LTR promoter or the human cytomegalovirus (CMV) IE promoter.

It may also be advantageous for the promoters to be inducible so that the levels of expression of the heterologous gene can be regulated during the life-time of the cell. Inducible means that the levels of expression obtained using the promoter can be regulated.

In addition, any of these promoters may be modified by the addition of further regulatory sequences, for example enhancer sequences. Chimeric promoters may also be

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used comprising sequence elements from two or more different promoters described above.

Polynucleotides according to the invention may also be inserted into the vectors described above in an antisense orientation to provide for the production of antisense RNA. Antisense RNA or other antisense polynucleotides may also be produced by synthetic means. Such antisense polynucleotides may be used in a method of controlling the levels of RNAs transcribed from genes comprising any one of the polynucleotides of the invention.

HOST CELLS

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Vectors and polynucleotides of the invention may be introduced into host cells for the purpose of replicating the vectors/polynucleotides and/or expressing the polypeptides of the invention encoded by the polynucleotides of the invention. Although the polypeptides of the invention may be produced using prokaryotic cells as host cells, it is preferred to use eukaryotic cells, for example yeast, insect or mammalian cells, in particular mammalian cells.

Vectors/polynucleotides of the invention may be introduced into suitable host cells using a variety of techniques known in the art, such as transfection, transformation and electroporation. Where vectors/polynucleotides of the invention are to be administered to animals, several techniques are known in the art, for example infection with recombinant viral vectors such as retroviruses, herpes simplex viruses and adenoviruses, direct injection of nucleic acids and biolistic transformation.

PROTEIN EXPRESSION AND PURIFICATION

Host cells comprising polynucleotides of the invention may be used to express polypeptides of the invention. Host cells may be cultured under suitable conditions which allow expression of the proteins of the invention. Expression of the polypeptides of the invention may be constitutive such that they are continually produced, or inducible, requiring a stimulus to initiate expression. In the case of inducible expression, protein

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production can be initiated when required by, for example, addition of an inducer substance to the culture medium, for example dexamethasone or IPTG.

Polypeptides of the invention can be extracted from host cells by a variety of techniques known in the art, including enzymatic, chemical and/or osmotic lysis and physical disruption.

Polypeptides of the invention may also be produced recombinantly in an *in vitro* cell-free system, such as the TnTTM (Promega) rabbit reticulocyte system.

ANTIBODIES

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The invention also provides monoclonal or polyclonal antibodies to polypeptides of the invention or fragments thereof. Thus, the present invention further provides a process for the production of monoclonal or polyclonal antibodies to polypeptides of the invention.

If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunised with an immunogenic polypeptide bearing an epitope(s) from a polypeptide of the invention. Serum from the immunised animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an epitope from a polypeptide of the invention contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art. In order that such antibodies may be made, the invention also provides polypeptides of the invention or fragments thereof haptenised to another polypeptide for use as immunogens in animals or humans.

Monoclonal antibodies directed against epitopes in the polypeptides of the invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced against epitopes in the

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polypeptides of the invention can be screened for various properties; i.e., for isotype and epitope affinity.

An alternative technique involves screening phage display libraries where, for example the phage express scFv fragments on the surface of their coat with a large variety of complementarity determining regions (CDRs). This technique is well known in the art.

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Antibodies, both monoclonal and polyclonal, which are directed against epitopes from polypeptides of the invention are particularly useful in diagnosis, and those which are neutralising are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies. Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the agent against which protection is desired.

Techniques for raising anti-idiotype antibodies are known in the art. These anti-idiotype antibodies may also be useful in therapy.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a target antigen. Such fragments include Fv, F(ab') and F(ab')₂ fragments, as well as single chain antibodies (scFv). Furthermore, the antibodies and fragments thereof may be humanised antibodies, for example as described in EP-A-239400.

Antibodies may be used in method of detecting polypeptides of the invention present in biological samples by a method which comprises: (a) providing an antibody of the invention; (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and (c) determining whether antibody-antigen complex comprising said antibody is formed.

Suitable samples include extracts tissues such as brain, breast, ovary, lung, colon, pancreas, testes, liver, muscle and bone tissues or from neoplastic growths derived from such tissues.

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Antibodies of the invention may be bound to a solid support and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

ASSAYS

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The present invention provides assays that are suitable for identifying substances which bind to polypeptides of the invention and which affect, for example, formation of the nuclear envelope, exit from the quiescent phase of the cell cycle (G0), G1 progression, chromosome decondensation, nuclear envelope breakdown, START, initiation of DNA replication, progression of DNA replication, termination of DNA replication, centrosome duplication, G2 progression, activation of mitotic or meiotic functions, chromosome condensation, centrosome separation, microtubule nucleation, spindle formation and function, interactions with microtubule motor proteins, chromatid separation and segregation, inactivation of mitotic functions, formation of contractile ring, cytokinesis functions, chromatin binding, formation of replication complexes, replication licensing, phosphorylation or other secondary modification activity, proteolytic degradation, microtubule binding, actin binding, septin binding, microtubule organising centre nucleation activity and binding to components of cell cycle signalling pathways.

In addition, assays suitable for identifying substances that interfere with binding of polypeptides of the invention, where appropriate, to components of cell division cycle machinery. This includes not only components such as microtubules but also signalling components and regulatory components as indicated above. Such assays are typically *in vitro*. Assays are also provided that test the effects of candidate substances identified in preliminary *in vitro* assays on intact cells in whole cell assays. The assays described below, or any suitable assay as known in the art, may be used to identify these substances.

According to one aspect of the invention, therefore, we provide one or more substances identified by any of the assays described below, *viz*, mitosis assays, meiotic assays, polypeptide binding assays, microtubule binding/polymerisation assays, microtubule purification and binding assays, microtubule organising centre (MTOC) nucleation activity assays, motor protein assay, assay for spindle assembly and function,

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assays for dna replication, chromosome condensation assays, kinase assays, kinase inhibitor assays, and whole cell assays, each as described in further detail below.

CANDIDATE SUBSTANCES

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A substance that inhibits cell cycle progression as a result of an interaction with a polypeptide of the invention may do so in several ways. For example, if the substance inhibits cell division, mitosis and/or meiosis, it may directly disrupt the binding of a polypeptide of the invention to a component of the spindle apparatus by, for example, binding to the polypeptide and masking or altering the site of interaction with the other component. A substance which inhibits DNA replication may do so by inhibiting the phosphorylation or de-phosphorylation of proteins involved in replication. For example, it is known that the kinase inhibitor 6-DMAP (6-dimethylaminopurine) prevents the initiation of replication (Blow, JJ, 1993, *J Cell Biol*122,993-1002). Candidate substances of this type may conveniently be preliminarily screened by *in vitro* binding assays as, for example, described below and then tested, for example in a whole cell assay as described below. Examples of candidate substances include antibodies which recognise a polypeptide of the invention.

A substance which can bind directly to a polypeptide of the invention may also inhibit its function in cell cycle progression by altering its subcellular localisation and hence its ability to interact with its normal substrate. The substance may alter the subcellular localisation of the polypeptide by directly binding to it, or by indirectly disrupting the interaction of the polypeptide with another component. For example, it is known that interaction between the p68 and p180 subunits of DNA polymerase alphaprimase enzyme is necessary in order for p180 to translocate into the nucleus (Mizuno et al (1998) *Mol Cell Biol*118,3552-62), and accordingly, a substance which disrupts the interaction between p68 and p180 will affect nuclear translocation and hence activity of the primase. A substance which affects mitosis may do so by preventing the polypeptide and components of the mitotic apparatus from coming into contact within the cell.

These substances may be tested using, for example the whole cells assays described below. Non-functional homologues of a polypeptide of the invention may also be tested

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for inhibition of cell cycle progression since they may compete with the wild type protein for binding to components of the cell division cycle machinery whilst being incapable of the normal functions of the protein or block the function of the protein bound to the cell division cycle machinery. Such non-functional homologues may include naturally occurring mutants and modified sequences or fragments thereof.

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Alternatively, instead of preventing the association of the components directly, the substance may suppress the biologically available amount of a polypeptide of the invention. This may be by inhibiting expression of the component, for example at the level of transcription, transcript stability, translation or post-translational stability. An example of such a substance would be antisense RNA or double-stranded interfering RNA sequences which suppresses the amount of mRNA biosynthesis.

Suitable candidate substances include peptides, especially of from about 5 to 30 or 10 to 25 amino acids in size, based on the sequence of the polypeptides described in the Examples, or variants of such peptides in which one or more residues have been substituted. Peptides from panels of peptides comprising random sequences or sequences which have been varied consistently to provide a maximally diverse panel of peptides may be used.

Suitable candidate substances also include antibody products (for example, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies and CDR-grafted antibodies) which are specific for a polypeptide of the invention.

Furthermore, combinatorial libraries, peptide and peptide mimetics, defined chemical entities, oligonucleotides, and natural product libraries may be screened for activity as inhibitors of binding of a polypeptide of the invention to the cell division cycle machinery, for example mitotic/meiotic apparatus (such as microtubules). The candidate substances may be used in an initial screen in batches of, for example 10 substances per reaction, and the substances of those batches which show inhibition tested individually. Candidate substances which show activity in *in vitro* screens such as those described below can then be tested in whole cell systems, such as mammalian cells which will be exposed to the inhibitor and tested for inhibition of any of the stages of the cell cycle.

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Polypeptide Binding Assays

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One type of assay for identifying substances that bind to a polypeptide of the invention involves contacting a polypeptide of the invention, which is immobilised on a solid support, with a non-immobilised candidate substance determining whether and/or to what extent the polypeptide of the invention and candidate substance bind to each other. Alternatively, the candidate substance may be immobilised and the polypeptide of the invention non-immobilised.

In a preferred assay method, the polypeptide of the invention is immobilised on beads such as agarose beads. Typically this is achieved by expressing the component as a GST-fusion protein in bacteria, yeast or higher eukaryotic cell lines and purifying the GST-fusion protein from crude cell extracts using glutathione-agarose beads (Smith and Johnson, 1988). As a control, binding of the candidate substance, which is not a GST-fusion protein, to the immobilised polypeptide of the invention is determined in the absence of the polypeptide of the invention. The binding of the candidate substance to the immobilised polypeptide of the invention is then determined. This type of assay is known in the art as a GST pulldown assay. Again, the candidate substance may be immobilised and the polypeptide of the invention non-immobilised.

It is also possible to perform this type of assay using different affinity purification systems for immobilising one of the components, for example Ni-NTA agarose and histidine-tagged components.

Binding of the polypeptide of the invention to the candidate substance may be determined by a variety of methods well-known in the art. For example, the non-immobilised component may be labeled (with for example, a radioactive label, an epitope tag or an enzyme-antibody conjugate). Alternatively, binding may be determined by immunological detection techniques. For example, the reaction mixture can be Western blotted and the blot probed with an antibody that detects the non-immobilised component. ELISA techniques may also be used.

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Candidate substances are typically added to a final concentration of from 1 to 1000 nmol/ml, more preferably from 1 to 100 nmol/ml. In the case of antibodies, the final concentration used is typically from 100 to 500 μ g/ml, more preferably from 200 to 300 μ g/ml.

Microtubule Binding/Polymerisation Assays

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In the case of polypeptides of the invention that bind to microtubules, another type of *in vitro* assay involves determining whether a candidate substance modulates binding of a polypeptide of the invention to microtubules. Such an assay typically comprises contacting a polypeptide of the invention with microtubules in the presence or absence of the candidate substance and determining if the candidate substance has an affect on the binding of the polypeptide of the invention to the microtubules. This assay can also be used in the absence of candidate substances to confirm that a polypeptide of the invention does indeed bind to microtubules. Microtubules may be prepared and assays conducted as follows:

Microtubule Purification and Binding Assays

Microtubules are purified from 0-3h-old *Drosophila* embryos essentially as described previously (Saunders, *et al.*, 1997). About 3 ml of embryos are homogenized with a Dounce homogenizer in 2 volumes of ice-cold lysis buffer (0.1 M Pipes/NaOH, pH6.6, 5 mM EGTA, 1 mM MgSO4, 0.9 M glycerol, 1 mM DTT, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 1 μg/ml pepstatin). The microtubules are depolymerized by incubation on ice for 15 min, and the extract is then centrifuged at 16,000 g for 30 min at 4°C. The supernatant is recentrifuged at 135,000 g for 90 min at 4°C. Microtubules in this later supernatant are polymerized by addition of GTP to 1 mM and taxol to 20 μM and incubation at room temperature for 30 min. A 3 ml aliquot of the extract is layered on top of 3 ml 15% sucrose cushion prepared in lysis buffer. After centrifuging at 54,000g for 30 min at 20°C using a swing out rotor, the microtubule pellet is resuspended in lysis buffer.

Microtubule overlay assays are performed as previously described (Saunders *et al.*, 1997). 500 ng per lane of recombinant Asp, recombinant polypeptide, and bovine serum albumin (BSA, Sigma) are fractionated by 10% SDS-PAGE and blotted onto PVDF

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membranes (Millipore). The membranes are preincubated in TBST (50mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween 20) containing 5% low fat powdered milk (LFPM) for 1 h and then washed 3 times for 15 min in lysis buffer. The filters are then incubated for 30 minutes in lysis buffer containing either 1 mM GDP, 1 mM GTP, or 1 mM GTP-γ-S. MAP-free bovine brain tubulin (Molecular Probes) is polymerised at a concentration of 2 ug/ml in lysis buffer by addition of GTP to a final concentration of 1 mM and incubated at 37°C for 30 min. The nucleotide solutions are removed and the buffer containing polymerised microtubules added to the membanes for incubation for 1h at 37°C with addition of taxol at a final concentration of 10 µM for the final 30 min. The blots are then 10 washed 3 times with TBST and the bound tubulin detected using standard Western blot procedures using anti-β-tubulin antibodies (Boehringer Manheim) at 2.5 µg/ml and the Super Signal detection system (Pierce).

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It may be desirable in one embodiment of this type of assay to deplete the polypeptide of the invention from cell extracts used to produce polymerise microtubules. This may, for example, be achieved by the use of suitable antibodies.

A simple extension to this type of assay would be to test the effects of purified polypeptide of the invention upon the ability of tubulin to polymerise in vitro (for example, as used by Andersen and Karsenti, 1997) in the presence or absence of a candidate substance (typically added at the concentrations described above). Xenopus cellfree extracts may conveniently be used, for example as a source of tubulin.

Microtubule Organising Centre (MTOC) Nucleation Activity Assays

Candidate substances, for example those identified using the binding assays described above, may be screening using a microtubule organising centre nucleation activity assay to determine if they are capable of disrupting MTOCs as measured by, for example, aster formation. This assay in its simplest form comprises adding the candidate substance to a cellular extract which in the absence of the candidate substance has microtubule organising centre nucleation activity resulting in formation of asters.

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In a preferred embodiment, the assay system comprises (i) a polypeptide of the invention and (ii) components required for microtubule organising centre nucleation activity except for functional polypeptide of the invention, which is typically removed by immunodepletion (or by the use of extracts from mutant cells). The components themselves are typically in two parts such that microtubule nucleation does not occur until the two parts are mixed. The polypeptide of the invention may be present in one of the two parts initially or added subsequently prior to mixing of the two parts.

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Subsequently, the polypeptide of the invention and candidate substance are added to the component mix and microtubule nucleation from centrosomes measured, for example by immunostaining for the polypeptide of the invention and visualising aster formation by immuno-fluorescence microscopy. The polypeptide of the invention may be preincubated with the candidate substance before addition to the component mix. Alternatively, both the polypeptide of the invention and the candidate substance may be added directly to the component mix, simultaneously or sequentially in either order.

The components required for microtubule organising centre formation typically include salt-stripped centrosomes prepared as described in Moritz *et al.*, 1998. Stripping centrosome preparations with 2 M KI removes the centrosome proteins CP60, CP190, CNN and γ -tubulin. Of these, neither CP60 nor CP190 appear to be required for microtubule nucleation. The other minimal components are typically provided as a depleted cellular extract, or conveniently, as a cellular extract from cells with a nonfunctional variant of a polypeptide of the invention. Typically, labeled tubulin (usually β -tubulin) is also added to assist in visualising aster formation.

Alternatively, partially purified centrosomes that have not been salt-stripped may be used as part of the components. In this case, only tubulin, preferably labeled tubulin is required to complete the component mix.

Candidate substances are typically added to a final concentration of from 1 to 1000 nmol/ml, more preferably from 1 to 100 nmol/ml. In the case of antibodies, the final

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concentration used is typically from 100 to 500 μ g/ml, more preferably from 200 to 300 μ g/ml.

The degree of inhibition of aster formation by the candidate substance may be determined by measuring the number of normal asters per unit area for control untreated cell preparation and measuring the number of normal asters per unit area for cells treated with the candidate substance and comparing the result. Typically, a candidate substance is considered to be capable of disrupting MTOC integrity if the treated cell preparations have less than 50%, preferably less than 40, 30, 20 or 10% of the number of asters found in untreated cells preparations. It may also be desirable to stain cells for γ -tubulin to determine the maximum number of possible MTOCs present to allow normalisation between samples.

Motor Protein Assay

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Polypeptides of the invention may interact with motor proteins such as the Eg5-like motor protein *in vitro*. The effects of candidate substances on such a process may be determined using assays wherein the motor protein is immobilised on coverslips. Rhodamine labeled microtubules are then added and their translocation can be followed by fluorescent microscopy. The effect of candidate substances may thus be determined by comparing the extent and/or rate of translocation in the presence and absence of the candidate substance. Generally, candidate substances known to bind to a polypeptide of the invention, would be tested in this assay. Alternatively, a high throughput assay may be used to identify modulators of motor proteins and the resulting identified substances tested for affects on a polypeptide of the invention as described above.

Typically this assay uses microtubules stabilised by taxol (e.g. Howard and Hyman 1993; Chandra and Endow, 1993 – both chapters in "Motility Assays for Motor Proteins" Ed Jon Scholey, pub Academic Press). If however, a polypeptide of the invention were to promote stable polymerisation of microtubules (see above) then these microtubules could be used directly in motility assays.

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Simple protein-protein binding assays as described above, using a motor protein and a polypeptide of the invention may also be used to confirm that the polypeptide of the invention binds to the motor protein, typically prior to testing the effect of candidate substances on that interaction.

Assay for Spindle Assembly and Function

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A further assay to investigate the function of polypeptide of the invention and the effect of candidate substances on those functions is an assay which measures spindle assembly and function. Typically, such assays are performed using *Xenopus* cell free systems, where two types of spindle assembly are possible. In the "half spindle" assembly pathway, a cytoplasmic extract of CSF arrested oocytes is mixed with sperm chromatin. The half spindles that form subsequently fuse together. A more physiological method is to induce CSF arrested extracts to enter interphase by addition of calcium, whereupon the DNA replicates and kinetochores form. Addition of fresh CSF arrested extract then induces mitosis with centrosome duplication and spindle formation (for discussion of these systems see Tournebize and Heald, 1996).

Again, generally, candidate substances known to bind to a polypeptide of the invention, or non-functional polypeptide variants of the invention, would be tested in this assay. Alternatively, a high throughput assay may be used to identify modulators of spindle formation and function and the resulting identified substances tested for affects binding of the polypeptide of the invention as described above.

Assays for DNA Replication

Another assay to investigate the function of polypeptide of the invention and the effect of candidate substances on those functions is as assay for replication of DNA. A number of cell free systems have been developed to assay DNA replication. These can be used to assay the ability of a substance to prevent or inhibit DNA replication, by conducting the assay in the presence of the substance. Suitable cell-free assay systems include, for example the SV-40 assay (Li and Kelly, 1984, *Proc. Natl. Acad. Sci USA* 81, 6973-6977; Waga and Stillman, 1994, *Nature* 369, 207-212.). A *Drosophila* cell free replication system, for example as described by Crevel and Cotteril (1991), *EMBO J.* 10,

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4361-4369, may also be used. A preferred assay is a cell free assay derived from *Xenopus* egg low speed supernatant extracts described in Blow and Laskey (1986, *Cell* 47,577-587) and Sheehan et al. (1988, *J. Cell Biol.* 106, 1-12), which measures the incorporation of nucleotides into a substrate consisting of *Xenopus* sperm DNA or HeLa nuclei. The nucleotides may be radiolabelled and incorporation assayed by scintillation counting. Alternatively and preferably, bromo-deoxy-uridine (BrdU) is used as a nucleotide substitute and replication activity measured by density substitution. The latter assay is able to distinguish genuine replication initiation events from incorporation as a result of DNA repair. The human cell-free replication assay reported by Krude, et al (1997), *Cell* 88, 109-19 may also be used to assay the effects of substances on the polypeptides of the invention.

Other In Vitro Assays

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Other assays for identifying substances that bind to a polypeptide of the invention are also provided. For example, substances which affect chromosome condensation may be assayed using the *in vitro* cell free system derived from *Xenopus* eggs, as known in the art.

Substances which affect kinase activity or proteolysis activity are of interest. It is known, for example, that temporal control of ubiquitin-proteasome mediated protein degradation is critical for normal G1 and S phase progression (reviewed in Krek 1998, *Curr Opin Genet Dev* 8, 36-42). A number of E3 ubiquitin protein ligases, designated SCFs (Skp1-cullin-F-box protein ligase complexes), confer substrate specificity on ubiquitination reactions, while protein kinases phosphorylate substrates destined for destruction and convert them into preferred targets for ubiquitin modification catalyzed by SCFs. Furthermore, ubiquitin-mediated proteolysis due to the anaphase-promoting complex/cyclosome (APC/C) is essential for separation of sister chromatids during mitosis, and exit from mitosis (Listovsky et al., 2000, *Exp Cell Res* 255, 184-191).

Substances which inhibit or affect kinase activity may be identified by means of a kinase assay as known in the art, for example, by measuring incorporation of ³²P into a suitable peptide or other substrate in the presence of the candidate substance. Similarly,

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substances which inhibit or affect proteolytic activity may be assayed by detecting increased or decreased cleavage of suitable polypeptide substrates.

Assays for these and other protein or polypeptide activities are known to those skilled in the art, and may suitably be used to identify substances which bind to a polypeptide of the invention and affect its activity.

Whole Cell Assays

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Candidate substances may also be tested on whole cells for their effect on cell cycle progression, including mitosis and/or meiosis. Preferably the candidate substances have been identified by the above-described *in vitro* methods. Alternatively, rapid throughput screens for substances capable of inhibiting cell division, typically mitosis, may be used as a preliminary screen and then used in the *in vitro* assay described above to confirm that the affect is on a particular polypeptide of the invention.

The candidate substance, i.e. the test compound, may be administered to the cell in several ways. For example, it may be added directly to the cell culture medium or injected into the cell. Alternatively, in the case of polypeptide candidate substances, the cell may be transfected with a nucleic acid construct which directs expression of the polypeptide in the cell. Preferably, the expression of the polypeptide is under the control of a regulatable promoter.

Typically, an assay to determine the effect of a candidate substance identified by the method of the invention on a particular stage of the cell division cycle comprises administering the candidate substance to a cell and determining whether the substance inhibits that stage of the cell division cycle. Techniques for measuring progress through the cell cycle in a cell population are well known in the art. The extent of progress through the cell cycle in treated cells is compared with the extent of progress through the cell cycle in an untreated control cell population to determine the degree of inhibition, if any. For example, an inhibitor of mitosis or meiosis may be assayed by measuring the proportion of cells in a population which are unable to undergo mitosis/meiosis and comparing this to the proportion of cells in an untreated population.

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The concentration of candidate substances used will typically be such that the final concentration in the cells is similar to that described above for the *in vitro* assays.

A candidate substance is typically considered to be an inhibitor of a particular stage in the cell division cycle (for example, mitosis) if the proportion of cells undergoing that particular stage (i.e., mitosis) is reduced to below 50%, preferably below 40, 30, 20 or 10% of that observed in untreated control cell populations.

THERAPEUTIC USES

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Many tumours are associated with defects in cell cycle progression, for example loss of normal cell cycle control. Tumour cells may therefore exhibit rapid and often aberrant mitosis. One therapeutic approach to treating cancer may therefore be to inhibit mitosis in rapidly dividing cells. Such an approach may also be used for therapy of any proliferative disease in general. Thus, since the polypeptides of the invention appear to be required for normal cell cycle progression, they represent targets for inhibition of their functions, particularly in tumour cells and other proliferative cells.

The term proliferative disorder is used herein in a broad sense to include any disorder that requires control of the cell cycle, for example, cardiovascular disorders such as restenosis and cardiomyopathy, auto-immune disorders such as glomerulonephritis and rheumatoid arthritis, dermatological disorders such as psoriasis, anti-inflammatory, anti-fungal, antiparasitic disorders such as malaria, emphysema and alopecia.

One possible approach is to express anti-sense constructs directed against polynucleotides of the invention, preferably selectively in tumour cells, to inhibit gene function and prevent the tumour cell from progressing through the cell cycle. Anti-sense constructs may also be used to inhibit gene function to prevent cell cycle progression in a proliferative cell. Another approach is to use non-functional variants of polypeptides of the invention that compete with the endogenous gene product for cellular components of cell cycle machinery, resulting in inhibition of function. Alternatively, compounds identified by the assays described above as binding to a polypeptide of the invention may

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be administered to tumour or proliferative cells to prevent the function of that polypeptide. This may be performed, for example, by means of gene therapy or by direct administration of the compounds. Suitable antibodies of the invention may also be used as therapeutic agents.

Alternatively, double-stranded (ds) RNA is a powerful way of interfering with gene expression in a range of organisms that has recently been shown to be successful in mammals (Wianny and Zernicka-Goetz, 2000, Nat Cell Biol 2000, 2, 70-75). Double stranded RNA corresponding to the sequence of a polynucleotide according to the invention can be introduced into or expressed in oocytes and cells of a candidate organism to interfere with cell division cycle progression.

In addition, a number of the mutations described herein exhibit aberrant meiotic phenotypes. Aberrant meiosis is an important factor in infertility since mutations that affect only meiosis and not mitosis will lead to a viable organism but one that is unable to produce viable gametes and hence reproduce. Consequently, the elucidation of genes involved in meiosis is an important step in diagnosing and preventing/treating fertility problems. Thus the polypeptides of the invention identified in mutant *Drosophila* having meiotic defects (as is clearly indicated in the Examples) may be used in methods of identifying substances that affect meiosis. In addition, these polypeptides, and corresponding polynucleotides, may be used to study meiosis and identify possible mutations that are indicative of infertility. This will be of use in diagnosing infertility problems.

ADMINISTRATION

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Substances identified or identifiable by the assay methods of the invention may preferably be combined with various components to produce compositions of the invention. Preferably the compositions are combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition (which may be for human or animal use). Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition of the invention may be administered by

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direct injection. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration. Typically, each protein may be administered at a dose of from 0.01 to 30 mg/kg body weight, preferably from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

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Polynucleotides/vectors encoding polypeptide components (or antisense constructs) for use in inhibiting cell cycle progression, for example, inhibiting mitosis or meiosis, may be administered directly as a naked nucleic acid construct. They may further comprise flanking sequences homologous to the host cell genome. When the polynucleotides/vectors are administered as a naked nucleic acid, the amount of nucleic acid administered may typically be in the range of from 1 µg to 10 mg, preferably from 100 µg to 1 mg. It is particularly preferred to use polynucleotides/ vectors that target specifically tumour or proliferative cells, for example by virtue of suitable regulatory constructs or by the use of targeted viral vectors.

Uptake of naked nucleic acid constructs by mammalian cells is enhanced by several known transfection techniques for example those including the use of transfection agents. Example of these agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example lipofectamTM and transfectamTM). Typically, nucleic acid constructs are mixed with the transfection agent to produce a composition.

Preferably the polynucleotide, polypeptide, compound or vector described here may be conjugated, joined, linked, fused, or otherwise associated with a membrane translocation sequence.

Preferably, the polynucleotide, polypeptide, compound or vector, etc described here may be delivered into cells by being conjugated with, joined to, linked to, fused to, or otherwise associated with a protein capable of crossing the plasma membrane and/or the nuclear membrane (i.e., a membrane translocation sequence). Preferably, the substance of interest is fused or conjugated to a domain or sequence from such a protein responsible for the translocational activity. Translocation domains and sequences for example include

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domains and sequences from the HIV-1-trans-activating protein (Tat), *Drosophila* Antennapedia homeodomain protein and the herpes simplex-1 virus VP22 protein. In a highly preferred embodiment, the substance of interest is conjugated with penetratin protein or a fragment of this. Penetratin comprises the sequence

RQIKIWFQNRRMKWKK and is described in Derossi, *et al.*, (1994), *J. Biol. Chem.* 269, 10444-50; use of penetratin-drug conjugates for intracellular delivery is described in WO/00/01417. Truncated and modified forms of penetratin may also be used, as described in WO/00/29427.

Preferably the polynucleotide, polypeptide, compound or vector according to the invention is combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration.

The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular patient and condition.

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The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

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EXAMPLES

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Generation and Identification of Lethal, Semi-Lethal and Sterile Third

Chromosome Mutants Having Defects in Mitosis and/Or Meiosis, and Second

Chromosome Mutants Having Defects in Imaginal Disc Development By P-Element

Insertion Mutagenesis

P-element mutagenesis

Transposable elements are widely used for mutagenesis in *Drosophila melanogaster* as they couple the advantages of providing effective genetic lesions with ease of detecting disrupted genes for the purpose of molecular cloning. To achieve near saturation of the genome with mutations resulting from mobilisation of the P-lacW transposon (a P-element marked with a mini-white gene, bearing the *E.coli lacZ* gene as an enhancer trap, and an *E.coli* replicon and ampicillin resistance gene to facilitate 'plasmid rescue' of sequences at the site of the P-insertion), *Drosophila* females that are homozygous for P-lacW (inserted on the X chromosome) are crossed with males carrying the transposase source $P(\Delta 2-3)$ (Deak et al., 1997). Random transpositions of the mutator element are then 'captured' in lines lacking transposase activity. Stable, or balanced, stocks bearing single lethal P-lacW insertions are made.

More than 41,000 lines are derived, of which approximately one-half are on the third chromosome. Originally some 3100 lethal or strong semi-lethal lines (in homozygous conditions) are identified. During preliminary characterisation unstable lines and clusters of the same mutation event are eliminated leaving 2460 lines to be characterised.

Screening for Mitotic and Meiotic Defects

About half of the mutants in the collection are embryonic lethals. We have carried out cytological screens of the 1155 lines that comprise late larval lethals, pupal lethals, pharate and adult semi-lethals for defective mitosis in the developing larval CNS. This has identified 69 mutations falling into 43 complementation groups that affect all stages of the mitotic cycle. The cytological screens involve examining orcein-stained squashed preparations of the larval CNS to detect abnormal mitotic cells. In lines where defects are

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identified, the larval CNS is subjected to immunostaining to identify centromeres, spindle microtubules and DNA for further examination. This leads to clarification of the mitotic defect.

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As a set of common functions are essential to both mitosis and meiosis, we then identify mutations resulting in sterility and failed progression through male meiosis. This involves examining squashed preparations larval, pupal or adult testes by phase contrast microscopy. We examine "onion stage" spermatids in the 519 pupal and pharate lethal lines and 463 adult "semi-lethal" and viable lines for variations in size and number of nuclei which provides an indication of whether there have been defects in either chromosome segregation or cytokinesis, respectively. A total of 54 lines of the 519 pupal and pharate lethal lines and 22 of the adult lines show such defects. However, another 67 lines show male sterility without having onion-stage defects. 12 lines showing onion stage defects have been scored as having mitotic defects in the independent cytological screen of squashed preparations of the larval CNS. Twelve further lines with onion stage defects show female sterility and of these, 10 show maternal effect mitotic defects in syncytial embryos. Thus greater than one half of the meiotic mutants scored appear to represent cell division functions specific to male meiosis or have targeted male germ-line specific enhancer elements, thus revealing their meiotic function but in this test not their mitotic function.

Further characterisation of testis preparations of each line by phase-contrast microscopy with and without staining with Hoechst to reveal DNA defined 6 broad categories of meiotic mutants:

8 mutants from the collection show defects in meiotic entry or at early stages in the first meiotic division (MF1-8).

18 mutants (15 complementation groups) show abnormal meiotic spindles (AB1-16). Mutants in this group almost invariably show an associated weak defect in cytokinesis, and 7 show a strong defect in spermatid differentiation. 3 of these mutants

also show mitotic defects in larval brains or in embryos derived from homozygous mutant mothers.

18 mutants (16 complementation groups) also show abnormal meiotic spindles that are strongly multipolar (MUL1-15). Three of these also show maternal effect mitotic abnormalities of multipolar spindles in syncytial embryos.

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4 mutants (3 complementation groups) show strong defects at all stages of spermatogenesis from the pre-meiotic stages to spermatid elongation stages (PL1-3). In this respect they resemble the *polo*¹ mutation.

4 mutants show segregation defects as indicated by spermatid nuclei of heterogeneous sizes (SEG1-4). The spindles appear normal but all have what are either chromosome bridges or lagging chromosomes. One of these also shows a maternal effect.

9 mutants (7 complementation groups) show predominant cytokinesis defects. Two complementation groups also have cytokinesis defects in mitotic cells in the larval brain.

In the Examples below, the designations MF, AB, MUL, PL, SEG or CK are included in the category description where available. Further phenotype information for each mutant described in the results section is provided in the "Phenotype" field. There is considerable overlap between these categories, and it will be of much interest to distinguish between mutants in which the primary defect results in secondary consequences, and mutants that affect more than one aspect of spermatogenesis, as for example appears to be the case with *polo* mutants (Sunkel and Glover, 1988; Carmena et al, 1998).

In the Examples, lines exhibiting mitotic and meiotic phenotypes are categorised generally into four categories:

Category 1: Failure to complete cytokinesis

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Category 2: Failure to enter M-phase

Category 3: Metaphase arrest

Category 4: Anaphase defect

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Category 5: Small Imaginal Discs (Block to Proliferation; see below)

Category 1 phenotypes are exhibited by mutations in Examples 1 to 14; while Category 2 phenotypes are exhibited by mutations in Examples 15 to 19. Category 3 phenotypes are exhibited by mutations in Examples 20 to 30, Category 4 phenotypes are exhibited by mutations in Examples 31 to 53. Mutations in Examples 54 to 74 exhibit a Category 5 phenotype.

Generation and identification of second chromosome mutants having small or no imaginal discs.

In the case of the second chromosome the flies used were from a second chromosome P-element collection established in Szeged, Hungary (Torok et al., 1993). The process of P-element insertion mutagenesis is essentially as described above. 15475 insertions were recovered, of which 2711 were lethal or semi-lethal. After elimination of clusters of identical mutants, 2399 lines representing 1748 independent lethal insertions were recovered. Lines were chosen from the second chromosome collection on the basis of having small or no imaginal discs, to indicate a disruption in cell cycle progression that leads to underdevelopment of the discs. All the second chromosome mutants referred to in the results section are noted under the "Phenotype" field as "second chromosome, small imaginal discs" and comprise Category 5.

Cytological Mapping of the P-Element Insertion Sites

The site of insertion of the P-element in each mutant line was determined by *in situ* hybridisation of P-element DNA to salivary gland polytene chromosomes as described in Saunders et al., 1989. Wandering third stage larvae were dissected and fixed as described and incubated with biotin-labeled DNA made from the *P-lacW* plasmid. After signal

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detection chromosomes were stained with Giemsa and examined by microscopy and signals indicating the presence of P elements were assigned to polytene chromosome bands referring to the Bridges map (Lefevre, 1976). In the majority of cases a single P element was detected, only 10% of lines having multiple (two or three) insertions. The site of insertion is given as the "Map Position" field in the results section (for example 77B)

Plasmid Rescue of P-Elements from Mutant Drosophila Lines

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Genomic DNA was isolated from adult flies by the method of Jowett et al., 1986, and plasmid rescue from the genomic DNA was performed according to Pirrotta et al., 1986. This allows the recovery of genomic DNA adjacent to the P-element which facilitates the identification of the site of P-element insertion and of genes which may be disrupted by the insertion. Essentially, genomic DNA derived from about 200 flies was digested with a restriction enzyme known to have a site within the P-element (EcoR1 or SacII for cloning sequences to the left of the element, or XbaI, BgIII, PstI or BamHI for sequences to the right of the element). The digested DNA was ligated overnight, and plasmids recovered by electroporation of the ligated DNA into *E.coli* XL1-blue competent cells. Appropriate primers from within the P-lacW sequence were used to determine the sequence of the genomic DNA flanking the element (on average, 400 bp of sequence were obtained). The rescue sequences are provided in the results section under the heading "Rescue sequence". Where more than one sequence was recovered, the orientation of each sequence is also given.

Sequence Analysis of P Element Insertion Lines

Sequences flanking the insertion site of the P-element were derived by P element rescue as described above. In some cases sequence was obtained from only one side of the insertion, while in other cases sequences were obtained from both sides of the insertion.

As a first step, each P element rescue sequence was used to search a total database of *Drosophila melanogaster* sequences (database of the Berkley *Drosophila* Genome project) using the BLASTN program (which compares a nucleic acid sequence with a nucleic acid database, (Altschul and Lipman 1990)) with default parameters.

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The search may identify a number of different types of match including *Drosophila* ESTs, known *Drosophila* genes and cloned genomic regions.

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The ability to identify genes already known to be essential for cell cycle progression using this approach was confirmed, in this example, by the rescue sequence obtained from line 1324/8 which mapped to the 77B locus which was used to search the database. A BLASTN search identified a number of matching *Drosophila* ESTs, a match with the known cell cycle regulatory gene *polo* and a cloned genomic region designated CSC: AC018188. These matches are recorded in the results sections under the field headings "*Drosophila* ESTs", "*Drosophila* gene hit" and "Genomic hit, Accession No.", respectively. Any entries under "*Drosophila* gene hit" are further annotated with "(BLASTN with Rescue sequence)" to show that the match was obtained using the rescue sequence rather than a *Drosophila* EST or genomic clone ORF (see below). Accession numbers of ESTs, genes and genomic clones are provided where known. Genomic clones designations often include the Genbank designation as part of a longer designation. However the Genbank designation is always the code beginning with "AC" and followed by six digits.

Where an EST was identified, this was subsequently used to search using the BLASTX program (default parameters) against databases of sequences from *Drosophila* and Homo sapiens (databases of the National centre for Biotechnology Information (NCBI), National Library of Medicine, National Institue of Health, USA). In the case of line 1104/16, the search identified a known human gene, phosphatidylinositol transfer protein (accession no. P48739) implying a novel function for this protein in cytokinesis. Human Homologues identified as a result of a BLASTX search using a *Drosophila* EST are shown in the results section under the heading "Human homologues" and annotated with "(BLASTX with EST)". *Drosophila* genes identified as a result of a BLASTX search using a *Drosophila* EST are shown in the results section under the heading "*Drosophila* gene hit" and annotated with "(BLASTX with EST)".

Where no *Drosophila* gene was identified using the initial BLASTN search but a matching genomic clone was found (a Bac or P1 clone often in excess of 100 kilobases), a

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20 kilobase segment of this genomic sequence (10 kilobases either side flanking the site of the P-element insertion) was subjected to a number of analyses.

If the rescue sequence matched sequences that lie within a known gene present within the genomic clone then these are presented under the heading "*Drosophila* gene hit (BLASTN with Rescue sequence". The known gene sequence was then used in a BLASTX search of a human database (NCBI – see above) to identify any human homologues. These are shown in the "Human homologue" field and annotated with "(BLASTX with *Drosophila* gene)".

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If the rescue sequence does not match any sequences that lie with a known gene within the genomic clone then the occurrence of ORFs within the 20 kilobase genomic segment was predicted using the Genscan programme (Burge and Karlin, 1997). Where the P-element was observed to be inserted into the coding region or within the 5' untranslated region (which we defined as within 2 kilobases of the predicted start of the coding region) we assume the P element to be capable of disrupting the expression of the predicted gene. Each predicted open reading frame (or predicted coding sequence) was then used to search *Drosophila* and human databases using the TBLASTN program (compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames against a nucleotide query sequence dynamically translated in all reading frames against a nucleotide sequence database dynamically translated in all reading frames) to determine whether the predicted open reading frame corresponded to a known gene. Typically, TBLASTX is only used when no matches are found using TBLASTN.

Where the TBLASTN search found a known *Drosophila* gene, then this is indicated in the results in the "*Drosophila* gene hit" field, annotated with "(TBLASTN with predicted ORF)". The *Drosophila* gene sequence was then typically used to search a human database (NCBI – see above) to identify any human homologues using BLASTX. These are shown in the "Human homologue" field and annotated with "(BLASTX with *Drosophila* gene)".

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Where the TBLASTN and/or TBLASTX search found a known human gene, then this is indicated in the results in the "Human homologue" field, annotated with "(TBLASTN (or TBLASTX) with predicted ORF)".

If the TBLASTN and/or TBLASTX search found no *Drosophila* or human genes, then it was assumed that the original ORF corresponds to a novel gene. If the TBLASTN search found no *Drosophila* genes but identified a human homologue, then it was assumed that the original ORF corresponds to a novel *Drosophila* homologue of a known human gene.

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Additional Sequence Analysis using the Annotated D. melanogaster Sequence (GadFly).

Rescue sequences were also used to search the fully annotated version of the Drosophila genome (GadFly; Adams, et al., 2000, Science 287, 2185-2195), using GlyBLAST at the Berkeley Drosophila Genome Projects web site to identify the genome segment (usually approximately 200-250 kb) containing the P-element insertion site. The graphic representation of the genomic fragment available at GadFly allows the 15 identification of all real and theoretical genes which flank the site of insertion. Candidate genes where the P-element is either inserted within the gene or close to the 5' end of the gene were identified. In GadFly, the *Drosophila* genes are given the designation CG (Complete gene) and usually details of human homologues are also given. In most cases, this data confirms the data derived from the sequence analysis procedure described above, 20 and in some cases new data is obtained. Where available both sets of data are included in the individual Examples described below. To identify further candidate human homologues, BLASTP (amino acid query sequence against amino acid database) searches with Drosophila sequences are used against the human genome project database and also 25 the Ensembl dataset. The Ensembl dataset comprises GeneWise gene predictions using a protein template where possible or Genscan followed by BLAST confirmation via protein, cDNA or EST hits. These are matched using WUBLASTP with default parameters (Altschul et al., 1990, J Mol Biol 215, 403-10). The results are filtered to contain only potential homologues. Only matches with the identity of more than 50% and length of more than 50 amino acids are included. 30

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Confirmation of Cell Cycle Involvement of Candidate Genes Using Double Stranded RNA Interference (RNAi)

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P-elements usually insert into the region 5' to a *Drosophila* gene. This means that there is sometimes more than one candidate gene affected, as the P-element can insert into the 5' regions of two diverging genes (one on each DNA strand). In order to confirm which of the candidate genes is responsible for the cell cycle phenotype observed in the fly line, we use the technique of double stranded RN interference to specifically knock out gene expression in Drosophila cells in tissue culture (Clemens, et al., 2000, Proc. Natl. Acad. Sci. USA, 6499-6503). The overall strategy is to prepare double stranded RNA (dsRNA) specific to each gene of interest and to transfect this into Schneider's Drosophila line 2 to inhibit the expression of the particular gene. The dsRNA is prepared from a double stranded, gene specific PCR product with a T7 RNA polymerase binding site at each end. The PCR primers consist of 25-30 bases of gene specific sequence fused to a T7 polymerase binding site (TAATACGACTCACTATAGGGACA), and are designed to amplify a DNA fragment of around 500bp. Although this is the optimal size, the sequences in fact range from 450 bp to 650 bp. Where possible, PCR amplification is performed using genomic DNA purified from Schneider's Drosophila line 2 as a template. This is only feasible where the gene has an exon of 450 bp or more. In instances where the gene possesses only short exons of less than 450 bp, primers are designed in different exons and PCR amplification is performed using cDNA derived from Schneider's Drosophila line 2 as a template.

A sample of PCR product is analysed by horizontal gel electrophoresis and the DNA purified using a Qiagen QiaQuick PCR purification kit. 1µg of DNA is used as the template in the preparation of gene specific single stranded RNA using the Ambion T7 Megascript kit. Single stranded RNA is produced from both strands of the template and is purified and immediately annealed by heating to 90 degrees C for 15 mins followed by gradual cooling to room temperature overnight. A sample of the dsRNA is analysed by horizontal gel electrophoresis.

3μg of dsRNA is transfected into Schneider's *Drosophila* line 2 using the transfection agent, Transfect (Gibco) and the cells incubated for 72 hours prior to fixation.

The DNA content of the cells is analysed by staining with propidium iodide and standard FACS analysis for DNA content. The cells in G1 and G2/S phases of the cell cycle are visualised as two separate population peaks in normal cycling S2 cells. In each experiment, Red Fluorescent Protein dsRNA is used as a negative control. In some cases the phenotype is confirmed by fixing cells on poly-lysine covered slides which are then stained for DNA using DAPI and for tubulin using an anti-tubulin antibody YL1/2 and appropriate fluorescent secondary antibody to visualise aberrant mitoses.

It should be noted that RNAi could not confirm phenotype in all cases. This is to be expected as the method relies on the ability of dsRNA to prevent new protein expression. Consequently, it is necessary that S2 cells express the specific cDNA of the gene in question, and also that the protein is turned over rapidly. It would therefore probably be difficult to sufficiently reduce levels of very stable proteins using this approach.

The layout of a typical entry in the results section is shown below. Not all fields

present in the actual results section contain information for each individual *Drosophila*line described.

TYPICAL RESULTS LAYOUT

Line ID - Drosophila line designation

Category - Description of phenotype

Reversion -R = revertant, NR = non revertant, ? = not determined

Map Position - according to the Bridges map (Lefevre, 1976).

Rescue ID

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25 Rescue Sequence

[nucleotide sequence]

Genomic hit, Accession No.

30 Associated ORF

GENSCAN_predicted_peptide [results of Genscan - amino acid sequence] GENSCAN predicted CDS [results of Genscan nucleotide sequence]

Drosophila Gene Hit

35 (BLASTN with rescue sequence)

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(TBLASTN (or TBLASTX) with predicted ORF) (BLASTX with EST)

Human Homologue

5 (BLASTX with *Drosophila* gene) (TBLASTN (or TBLASTX) with predicted ORF) (BLASTX with EST) **Drosophila EST**

10 Annotated *Drosophila* genome genomic segment Annotated *Drosophila* genome Complete gene candidate Human homologue of Complete gene candidate

Putative function Derived from homologies or Drosophila experimental data

Confirmation by RNAi Description of Facs analysis DNA content profile

A specific example is as follows:

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Line ID 1324/8

Category Mitotic defects in brain: metaphase arrest

(overcondensation, some circular chromosomes, no anaphases, very high mitotic index, metaphase (or less aligned) with bipolar

spindle, no CP190 staining)

Reversion R **Map Position** 77B

Rescue ID B1E

30 Rescue Sequence

GTTTTGCCCATCGATTGCACGAAAACCAAGCACAAAGCGGAGAACGCGCCGA AACCGTTCGATTTTTTAAATGCCAAAATGAATTGGACGTGAAGCGTCAGCTGA ATTGGTGTGCCCGTTTCGGTGGCTATCGCACACTTTCTGGTATTTATCGCGGTA TTTTGTTGAGTGTTGAACAACAAATTCTATGGCCGTTACCCTTTTGAATTTACT

- TACTGGCGTTTACTCTGTTCGAATTGAGCGCAATATTTTTTCCTATTGCTCTGC GCAACACTGTGTTTTAACCGCTATTTATTTGAAAATCTACAAAAACTAACCGTT TACATTTTTGAAATTTCCAAAAAGGGTTTTCCATAAATTGAGTTTTACTAAAACC AGTCCAACGGTCCAACTTTATATTGTTAGAAGCCCCTTTTCCTAATTTGAATTG GCTTGCAAACGTTTTCCTGAATTTAAAAAATACTGCCACCCTTGTTAATTGCAGG
- 40 TTTTCCGAATCCCTGATTTGTTTTTAAAAAGAAAATTTATTAGAAACAGCTA TCTCAACC

Genomic hit, Accession No. CSC:AC018188

Drosophila Gene Hit Polo (X63361)

45 **Human Homologue** BLASTX PLK-1 (P53350)

Drosophila EST several including LD11851 (AA392613) which match polo

Annotated *Drosophila* genome genomic segment AE003514

56

Annotated Drosophila genome Complete gene candidate CG12306

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Human homolog of Complete gene candidate 1e-169 1709658 P53350

PLK1_HUMAN

SERINE/THREONINE-PROTEIN KINASE PLK

(PLK-1)

Putative function Serine/threonine kinase known to be required for mitosis

10 **Confirmation by RNAi** Reduced G1 and G2/M peaks indicating fewer cycling cells, microscopy analysis of DNA and tubulin staining identified

monopolar spindles characteristic of polo mutation in

Drosophila.

WO 01/72774

PCT/GB01/01297

57

CATEGORY 1: FAILURE TO COMPLETE CYTOKINESIS

Example 1 (Category 1)

Line ID 1031/14

5 Category Mitotic defects in brain: cytokinesis defect

(polyploidy)

Reversion R **Map Position** 74B

10 Rescue ID 2A3B

Rescue Sequence 1

- 15 GACTAATGTGTTTAAATGTAACTTACACTAGTAACAGATCCCCATTAATAAAA GCCAAACTCTAAAATTCTGCCACAAGTACTATTTCTCACGTAACACCTTACTA ACGGATTTCACATGATATCTACGACAAGAAACTGTTTGCTGATATAAAATTGC TATCACCGCTTTCCGTAAACACTTTTACACTGATGGATTACAAGTTCAATTAAT ACATCAACTTACCTTAACAATTTAAGACAACTAACACTCCCACAATTTAATT

Rescue ID 2A3S

- 25 Rescue Sequence 2
- 30 CGGCAACACACTCTGGACTTGCAGCCGCTCCTGGCGGAGAGCGATGTCGGAA ACAGGGAGCTGGAGGAGAAGATGGGCGGATCGGCGGATCGCTCCTGCTC GATGGATCCGGTTCGAAGGAGCTGAGTCACCGGGAACGCGAGGACTCGGCGTT GTTCGTCAAGAAGATCGGGAGCGCCTTGTTCTATGGCTTGTCCTCCTTCATGATT ACGGTGGTAAACAAGACGGTGCTTACCTCCTACCACTTCCCTCGTTCCTGTTCC
- 35 TCAGCCTCGGGCAACTTACTGCTAGCATTGTGGTCCTGGGCATGGGCAAAGCGC CTGAAAATGGTGAACTTTTCCCCTTTTGCAGAGGAATACCTTCGCCAAGATCTTT CCGCTGCCACTGATATTTCTGGGAAACATGATGTTTGGACTGGGTGGCACAAAA ACCTTGAGTCTGCCCATGTTCGCAGCCCTACGAC
- 40 Genomic hit, Accession No. AC019515

Associated ORF

Genscan ORF1 predicted sequences:>15:31:57|GENSCAN predicted_peptide_4|373_aa

58

MSMSRGGNTTLDLQPLLAESDVGNRELEEKMGGSADRSSLLDGSGSKELSHRER EDSALFVKKIGSALFYGLSSFMITVVNKTVLTSYHFPSFLFLSLGQLTASIVVLGMG KRLKLVNFPPLQRNTFAKIFPLPLIFLGNMMFGLGGTKTLSLPMFAALRRFSILMT MLLELKILGLRPSNAVQVSVYAMIGGALLAASDDLSFNMRGYIYVMITNALTASN GVYVKKKLDTSEIGKYGLMYYNSLFMFLPALALNYVTGNLDQALNFEQWNDSV FVVQFLLSCVMGFILSYSTILCTQFNSALTTTIVGCLKNICVTYLGMFIGGDYVFSW LNCIGINISVLASLLYTYVTFRRKRAPDKQDHLPSTRGENV

>15:31:57|GENSCAN predicted CDS 4|1122 bp

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10 atgagtatgtegegeggeggeaacacacactetggacttgcagecgeteetggeggagageggtgtggaaacagggagetgga ggagaagatgggcggatcggcggatcggtcatcgctgctcgatggatccggttcgaaggagctgagtcaccgggaacgcgag gactcggcgttgttcgtcaagaagatcgggagcgccttgttctatggcttgtcctccttcatgattacggtggtaaacaagacggtgc ttacctcctaccacttccctcgttcctgttcctcggctcgggcaacttactgctagcattgtggtcctgggcatgggcaagcgcct gaaattggtgaactttcccctctgcagaggaataccttcgccaagatctttccgctgccactgatatttctgggaaacatgatgtttg 15 gactgggtggcacaaaaaccttgagtctgcccatgttcgcagccctacgacgcttctctatcctgatgaccatgctgctggagctca agatectgggaetgegaettegaatgeggtteaggteagegtataegeaatgateggtggagegetgetggeegeetetgatga tetgteetteaacatgaggggetacatetatgtgatgateactaacgeettgacegeetegaatggegtatatgtgaagaaaaaacte 20 ctategta cageaceate tgtgcaegea atteaacteggegetgae caecaceattgtgggatgeetgaaaaa catetgegtaaeatatetgggeatgtteattggaggegaetaegtettetegtggeteaactgtattgggateaacateagegtgetggetagtetgetet acacgtacgtcacttttcggcggaagcgggctcccgataagcaggaccacttgcccagcacccgcggggagaatgtctag

Human Homologue (TBLASTN with ORF1): KIAA0260 gene (D87449) and putative

Sqv-7-like protein (AJ005866)

Drosophila EST CK00510 (AA140776)

Annotated *Drosophila* genome genomic segment AE003524 Annotated *Drosophila* genome Complete gene candidate CG3874 – novel glucose-6-

30 phosphate transporter

Human homologue of Complete gene candidate EMBL:D87449 protein

KIAA0260_id:BAA13390 gi:166578 Similar to a C.elegans protein encoded in

cosmid C52E12 (U50135) and

Ensembl predicted gene ENSG00000024527 Clone:AL133320

Contig:AL133320.00001

8.10E-95

Putative function Sugar modification protein similar to proteins involved in

Drosophila cytokinesis and signalling

Confirmation by RNAi Marked increased G1 and S peak indicating mainly arrest in

59

Example 2 (Category 1)

Line ID 1066/5

Category Male semi-sterile, Meiotic defects in testis: cytokinesis defects,

segregation defects.

(Seg-01/62)

Reversion ? Map Position 89B

10 Rescue ID F9E

Rescue Sequence

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Genomic hit, Accession No. CSC:AC019750

25 Associated ORF

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>16:04:57|GENSCAN_predicted_peptide_4|418_aa
MKPIPNESKGTLAAVGDATVVHDVCTLFAVELDPYLRSSMGMRTRRAQSGALLL
QLLAVADGGFAAHICACKCRLRLPHVTCCCNRNPFKATAKAKGQAVSSTKPNQL
CFHGCCGWIITTKGETFTENSPSIMSGFAWERHSLGECVVVAGTEQILLIGRTLIGR
MSHTQTDSTSPFVVDCHSQLCGSKCKCICVSVGFCVRPSCQRFDMKIVWANLAM
QKRFLLGAAIADMCCRNSVIWCKLQLDPVKPIDERADGSGLALVTKVCDNNNIV
HYVVVAGVTGSQSRSRLQPLRSGQNESTEQWPRTKGGEGGFNNNSRNNKHSAPT
QEQQELWQKQLLQDQRDDCHASGSFQSASFAETRSFTFDDTTAHSEFCFRTRAEK
RRILVLLETSIKLKPDKYATSGHTRRCAIGLLHSII

>16:04:57|GENSCAN predicted CDS 4|1257 bp

60

Drosophila Gene Hit rescue sequence: mitotic heterochromatin fragment clone CH(2)6 (L36595) and subtelomeric heterochromatin repeats (L03284).

TBLASTN with ORF1: nebula (nla) (AF147700)

Human Hamalagua PLASTN with polyals: Down Syndrome candidate region 1 like

10 **Human Homologue** BLASTX with nebula: Down Syndrome candidate region 1-like protein 2 (AF176117)

Drosophila EST rescue sequence: CK01138 (AA141069)

Annotated *Drosophila* genome genomic segment AE003712
Annotated *Drosophila* genome Complete gene candidate CG6072 - nebula
CG6046 - sap18

Human homologue of Complete gene candidate CG6072- 8e-36 'ZAKI4 a thyroid 20 hormone responsive gene in human skin fibroblasts' also known as DOWN SYNDROME CANDIDATE REGION 1-LIKE 1; DSCR1L1 EMBL:D83407 25 protein id:BAA11911 gi:143504 CG6046- 3e-45 2108210 (U96915) sin3 associated polypeptide p18 [Homo sapiens] and gi5032067 30 C7E479FFE9CA5774 |ref|NP 005861.1| sin3-associated polypeptide, 18kD [Homo sapiens] (1.90E-43)

35 **Putative function** Nebula unknown function, Sap18 transcription factor

Confirmation by RNAi Both show reduction in G1 and G2/S peaks indicating fewer cycling cells

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Line ID

234/50

Category

Meiotic defects in testis: cytokinesis defects, abnormal spindles.

(Ab-02/12)

Reversion

Map Position

R 89B

Rescue ID

2C5E

Rescue Sequence

Drosophila EST

rescue sequence: CK01138 (AA141069)

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All other entries as for 1066/5.

WO 01/72774

62

PCT/GB01/01297

Example 3 (Category 1)

Line ID

1104/16

Category

Mitotic defects in brain: cytokinesis defect

(no overcondensation of diploids, high polyploidy)

Reversion

R

Map Position

92A

Rescue ID

B₅P

10 Rescue Sequence 1

CTCCGGACACGCAGTAGCTAAATAACAAACTCATTACTAGTATATTACTGCCG CCGATTTGCAAGCGCGTACCGATCCCGATACCAGGCCAATCGCACTCCCCAGT TGTACGTCATCACTTAAGTAATAAATCAGCGGCAAATCGCATAAATTGCTATT GTGTGCATATGACTCGTGCGTTTAGCCGACAATTGGAGAAAAAGCATTACCAA TCCCAATTGGCTAACTAAACTAAAGTTGGCTTGGCCAAACATAAACAAAAAGT GCGGGCGCAGCGATTTGGCAGCGAAACATATACACCAAAGCGCTATTGGCAG ATATATATGTAGATTAAATATAGAAAGTGCGTGCGAAGGTTAAGAGTCGAGT GCAAGTGCATTTATATTTGGAAATAATAAATGCTACAAT

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Rescue ID B₅E

Rescue Sequence 2

GTCCGGAGCGAGCTAAAGTTCGATGTTCGTGCAAAACACTTCGATTCCGATA GGCGGATGCTATCGATTTCGGCGATGCCCGTTGGTCACACTTGGTGGTGGGCG ${\sf CTGCCGTCGCCGACTATCGATAGCACAAGCGGGTTATTTAGGTGTGCGCAGC}$ TTGTAAGGGTGACTCATGCTGTTAAAAATTATTATAAAAAGTTAATGAATATAA TATAGTTATAATAAAATTATATAAATCTATAAGATCAAAGATCATCAGTTA TCATTTATCATTTGATTATGAAAAACAAGAACAGAACAAGATTTAATAGG TTTTTGAAATGTGAAAATGTGGGTTACCCCCAATTCTTATTCGAAATTAAATAA CCTAAAGAACAGTTATACACAGATAGGTAATTTGCACATAAGCCAAATTTTGT CTAGAATTCCGCGGAATTAATTCTTGAAGACGAAAGGGCCTCCGTGATACGCC TATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTA

Genomic hit, Accession No. AC006589

35 **Associated ORF**

Genscan: ORF1 predicted sequences

>/tmp/aaaaainga|GENSCAN predicted peptide 2|850 aa

MATRGANVIWFRHGLRLHDNPALLAALADKDOGIALIPVFIFDGESAGTKNVGY NRMRFLLDSLQDIDDQLQAATDGRGRLLVFEGEPAYIFRRLHEQVRLHRICIEQDC **EPIWNERDESIRSLCRELNIDFVEKVSHTLWDPQLVIETNGGIPPLTYQMFLIRCTH**

- 40 HNGDVNGDEDTGEGEGTGGRIDWAKEGACWRAGNSDEQECQACQSVSSVIMM VLQYSNNPAHHCQLLECLMTLKHNVVKDILCVVAYGTAVSRTSAAKLLFYYWP AFNANLFDRKVLLSKLTNDLVPFTCQREHCPNSGNAEAAKVCYDHSISIAYAPDC PPPLYLCIECANEIHREHGSLEFGDILHPMQQVSMVCENKNCRSNEKSAFSICFSTE
- CASFNGNHPIRYCSQCHSNRHNSRRGGDHVVHRSLQPAWQMDPEMQMHMVESV 45 VSLLREAKPLNFEPGKESSSSESKKNGSGITADNISLEERQRLGRYGIWLLVGRCTP

TADTPVEVLGRILSMLFHWFHVTAYSYDGFISCLVPHPPEYARVGGHWETLASRT SHLKEGLQRLICLVPYEVITSEIWDYVMPHWMEAITNDVAEKELNELKIVLSKILD PEMSPLGFDAKTMYNFVAIRFEKTTAKVQQQALHWLQILTKLEILIPLVQLFAMF GDGVRIMKYGIQHELMREKDAQSQSLAKAPKTPCKESKETKADMANPPRPPVVE DDSGNTSAISDDEAPTNRHTEFSTDAEHNLTCCILMLDILLKQMELQDVEQHMGI HTSVCENVSRLIKCMVTAARVGLSSHVCALKVPIEDIIEEEKSSRKSPPESDKEKTR DRDVSLSMAPLPIPLGPLGGFADP

5

>/tmp/aaaaainga|GENSCAN_predicted_CDS_2|2553_bp 10 atggccacgegagggcgaatgtgatttggtttcgccatggattgcgcctccatgataatcccgctctattggccgccctcgccgataaggatcagggtatagccctaattcccgttttcatattcgatggagagagtgcaggtacc aagaatgtgggttacaatcggatgcgtttcctcctggactcgttgcaggacatcgatgatcagctacaggcggcaactgatggacg tggacgcctcctggtcttcgagggcgaaccggcttatatcttccgccggctacatgagcaagtgcgtctgcacaggatttgcatag agcaggactgcgagccaatttggaatgagcgcgatgaaagcatccgttctctatgtcgggagctgaatatcgactttgtcgagaag 15 gtateacacacgetttgggateegeaattggtgattgagaccaattggtggcatteeaccgetgacctaccaaatgtteetgatacgetgcacgcaccaca at ggagat gt gaat gg ggat gaggat ac gg gagaa ggagaa ggaacc ggc ggaa ggat cgact ggc taaggaaggggcetgttggaggggggaaactccgacgaacaggaatgtcaggcctgccaatcagtgtcctcggtcatcatgatg gtgctccagtactccaacaatccagcgcatcattgccagctcctggagtgcctgatgactcttaagcacaatgtcgtcaaggacatc 20 tgttegategeaaagteetaeteteeaaaetaaceaatgaeetagtgeeetteaeetgeeaaegggageaetgteegaaeteeggg tegagtgegeeaacgagatteategggageaeggaageetggagtteggegaeattetgeateeeatgeageaggtategatgg tgtgcgaaaacaagaactgtcgctccaacgagaagtccgccttctccatctgcttctccacggagtgtgccagcttcaatggcaac catecgatecgetactgeagecagtgecacagtaataggeacaatteeeggegaggtggegateaegtggtecategeagtetge25 agcccgcctggcagatggatccagagatgcagatgcacatggtggagtcggtggtaagccttctgcgagaggcgaagccactaaacgccagagactgggacgctatggtatctggctactggtgggtcgctgtacacccactgcagatactcccgtagaagttctggg gtatgcccgtgttggaggccactgggagaccttggcgtcgcgaacaagccacttgaaagagggtcttcagcggcttatatgcctg 30 aggaact gaacgag ctgaag at tgtgctcag caag at cctcgatccggaaat gtcgcctct gggcttt gatgccaaaaccat gtacaactttgtggccattcgatttgagaagacaacggcaaaggtgcagcagcaggcactccactggctgcagatcctcaccaagctgg agatteteatteeaetggteeagttgttegeeatgtteggegatggtgttegeataatgaaataeggeateeageaegagetgatgeg cgagaaggatgcccaatctcagtccttggccaaggctcccaagaccccttgtaaagagagcaaggagaccaaagcggatatg 35 gcca at ccgcccagg cct cctgtt gtcgagg at gactct ggta at acgtct gccattt cgg at gacgagg cccac gaat cgt call the second seccacggaattctccacggatgctgagcacaatctcacctgttgcatcctcatgctggacatacttctgaagcaaatggaactacagga egtggageageacatgggcatecataegagtgtetgegagaacgteteeaggetgateaagtgcatggtcaetgeagetegagt gggtctcagtagtcatgtctgcgccttaaaggttcccatcgaggacatcattgaggaagaaaagtcctcgcgcaaatctccacccg a at ccga caaggaa aa agacccgt gat cga gat gttt ccctct cgat ggctccactacccattccgct gggacctt taggaggat tt gat can be a compared to the compared transfer of the compared tra40 cagacccttaa

Human Homologue BLASTX with EST: Phosphatidylinositol transfer protein (P48739)

45 *Drosophila* EST SD01527 (AI530804), GH18602 (AI387906)

Annotated *Drosophila* genome genomic segment

AE003725

64

Annotated *Drosophila* genome Complete gene candidate CG5269 – vib PIP transfer protein

Human homologue of Complete gene candidate 1e-90 1346772 P48739

PPI2 HUMAN

PHOSPHATIDYLINOSITOL TRANSFER PROTEIN BETA

ISOFORM

10 **Putative function** phosopholipid transporter involved in lipid metabolism

Confirmation by RNAi Slight reduction of G1 and increase in G2/M peaks

indicating arrest in G2/M

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65

Line ID 418/32

Category Meiotic defects in testis: cytokinesis defects. Dark bands in eyes,

dominant.

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Reversion ? **Map Position** 69C

Rescue ID G2E

Rescue Sequence

Genomic hit, Accession No. AC006589

20 *Drosophila* EST SD01527 (AI530804), GH18602 (AI387906)

Rest of results same as line 1104/16

66

Example 4 (Category 1)

Line ID

1285/1

Category

Meiotic defects in testis: cytokinesis defects

5 Reversion

?

Map Position

85D1-5

Rescue ID

D8E

Rescue Sequence

Genomic hit, Accession No. CSC:AC014256

20 Associated ORF

Genscan ORF1 predicted sequences

>/tmp/aaaaakfaa|GENSCAN predicted peptide 1|702 aa

MIQRCVVLLWIVCFCDLFLGLLFLKRKRNAHTPPPPPQFTTYRHLLCYCFRNGEIM ANICLSRLSVLEEIVLLLRVPCAFYFVDYYYVPCLLSVLSESFLYHDQLKVFNRTK

- 25 QQHQQQQQQQQLYQQHQQQQQHYGPPPPYFQQLHQQHQQQQQQQQQQQ HQQHMKFLGGNDDRNGRGGVGVGTDAIVGSRGGVSQDAADAAGAAAAAVG YVFQQRPSPGGVGVGGGGGVPGVGAVGSTLHEAAAAEYAAHFAQKQQQT RWACGDDGHGIDNPDKWKYNPPMNPANAAPGGPPGNGSNGGPGAIGTIGMGSG LGGGGGGAGGGNNGGSGTNGGLHHQSMAAAAANMAAMQQAAALAKHNHMI
- 30 SQAAAAVAAQQQHQHPHQQHPQQQQQQQQQQQQQHPHHLMGGGNGLGNGNG LGIQHPGQQQQQQQQQQQQHPGQYNANLLNHAAALGHMSSYAQSGGSMYDH HGGAMHPGMNGGMPKQQPLGPPGAGGPQDYVYMGGQTTVPMGAAMMPPQNQ YMNSSAVAAANRNAAITTSTAKKLWEKSDGKGVSSSTPGGPLHPLQIPGIGDPSS VWKDHTWSTQGENILVPPPSRAYAHGGASDTSNSGNAGILSPRDSTCAKVVEYVF
- 35 SGSPTNKDSSLSGLEPHLRNLKFDDNDKSRDDKEKANSPFDTNGLKKDDQVTNSN GVVNGIDDDKGFK

>/tmp/aaaaakfaa|GENSCAN_predicted_CDS_1|2109_bp

WO 01/72774

67

ggtggcggtgtgccaggggtcggagccgtaggctcgaccttgcacgaggccgccgccgagtacgccgccactttgccc agaagcaacagcagaccgatgggcgtgcggcgacgacggccatgggatcgataacccggacaaatggaagtacaatccgc atgggcagcggattgggtggtggtggcggcggagctggcggagaaataatggcggctctggtacgaatggcggtctgc atcatcaatcgatggccgctgcagctgcgaatatggcagccatgcaacaggcggcggcggttggccaagcacaatcacatgatat geaggegeagaaceaggggateeacateacettatgggeggtggcaatggactgggcaacggcaatggattgggcatacaa catcceggccagcaacagcagcagcagcaacaacagcagcagcaacatcceggccagtacaacgcgaatctgettaacc atgcggctgccttgggtcacatgtcatcttatgcccaatcgggtggcagcatgtacgaccatcatggtggagccatgcacccggg cagaccactgtgcccatgggagccgcaatgatgccgccacagaatcaatatatgaacagctctgctgttgcagctgccaatcgga atgcagcgattaccacatccactgccaagaaattgtgggagaaatccgatggcaagggcgtatcctcgagcactcccggtggac cgttgcatcccctgcagatccccggcatcggggatccctcctcctgtgtggaaggatcacacctggtccacacagggcgagaatatattggtgcgccccctcgcgagcctacgcccatggaggcgctccgatacttcaaacagcggcaatgcgggcatactgagtcc gcatttgcggaatctaaagtttgacgacaacgataagtcacgcgacgataaggagaaagcaaactctccgtttgacacaaacggtt tgaagaaagacgatcaggtcacaaactcaaatggtgttgtcaacggcattgacgatgacaagggcttcaagtga

Human Homologue

Drosophila Gene Hit TBLASTN of ORF1: pumilio protein (L07943)

TBLASTX with pumilio: Soares fetal heart NbHH19W Homo sapiens cDNA clone (W77820)

Annotated Drosophila genome genomic segment Annotated Drosophila genome Complete gene candidate CG9755 – pumilio RNA

AE003681

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Human homologue of Complete gene candidate

1e-154 1944416 dbi|BAA19665| (D87078) similar to D.melanogaster pumilio protein (S22026)

30

Putative function

Putative RNA binding protein which is localised to the cytoplasm. Wild-type allele of pum involved in development of the abdomen (embryos) and of the imaginal discs (larvae or pupae), perhaps having a function in signal transport.

35

Confirmation by RNAi

Only wild type profiles observed

68

Example 5 (Category 1)

Line ID 1389/1

Category Meiotic defects in testis:segregation defect, cytokinesis defect

(Ck-09/32)

Reversion NR **Map Position** 93B4-8

Rescue ID 2C9P

10 Rescue Sequence 1

CAAGAAGCAGCAGCAGCAGCAGTAGAAATAGCAAAAGGAGGCAGCAAC AACAATAAGCTAGAGAAACCGCCAGCAGCAGCCCCCTAATAAAGAGCAGAGA AAAAAATGAGTTCAAGTTGTGAAAGGTGTGTGCCGTTACACTACAAACTACAA CACCACCATCAGCGGCAGCAAAGAAATACAACAACAAATACGGCAATCTCCA 15 GACAACGCGAATGTCGAAATTGTGTATACAATTTATTAAGAAAGCAAGAGCA GCAACAACAATGACCAGCTGCAGTTCATCAGCGGTGTCCTCCTGAATGCCGCT GTCGTCGTTGGTGTCTGCCACCGGCGGTTCCTCAATAATAAGGGCAGGAGGAG ${\tt CTGCTTAGGTGCACACAATGTAGTTTGGCTTGGTGAATGCTTCTCTTTTTGTTG}$ ${\tt CTGCTGGCGCATACGTTCCTCTTCTCCCCTCATGATCTCAGTTGTCTGCATCGA}$ 20 TGAGCCGCCACCAACGGTGGCTTCTTCTGCTCCTCTTTTGGCAACGGACTGCTG CAGTCTTGCCAGAATTTTTCCTAAAATACTGAGCTTCAACTTGGTCTGGT AATGGTATACCATAAGCCATGGACTTGATGCCCCTACAAAGCTCTGTGATTTG **AAATGGGATGCA**

25

30

35

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Rescue ID 2C9E

AAGTAGTTCTCTTATGGATGCATC

Rescue Sequence 2

40 Drosophila EST

several including LD10379 (AA816796)

Annotated *Drosophila* genome genomic segment AE003733

Annotated *Drosophila* genome Complete gene candidate CG3421 - novel protein with weak homology to myosin

69

Human homologue of Complete gene candidate

5

Ensembl predicted

Gene:ENSG00000071333

Clone:AC022505

Contig:AC022505.00011 5.60E-37 (predicted protein with Core domain in kinesin

and myosin motors ENSG00000087179)

10 Putative function Possible novel motor protein involved in cytoskeleton organization

Confirmation by RNAi Marked reduction of G1 and G2/M peaks indicating fewer cycling cells

70

Example 6 (Category 1)

Line ID

293/9

Category

Mitotic defects in brain: cytokinesis defect

5

(no overcondensation of diploids, very high polyploidy)

Reversion

NR

Map Position

66B

Rescue ID

2G5E

10 Rescue Sequence

20 T

35

40

15

Genomic hit, Accession No. AC008303

Associated ORF

25 Genscan ORF1 predicted sequences >20:53:38|GENSCAN_predicted_peptide_3|261_aa MMDNDDALLNNGGPQSGAETVYGTEDNNMVMSEKCRIFPATQRTGFVGATFSG VLLLDLGALQHCDVIRIDVNIATLEQIKRERQEELAARERIRAQIAADRAEQAQRF NTPDISSTTNSVAATAASNVITTDASVSSVDETRLQIRLPGGIQRTKSFPAGEVLAT VRVYVRNEMLAASDVRDFTLATSYPRREFQTEDEVKTLNELNLVPNAVVLVLTK

30 EQVNPADDQTAKRSASTKRTKTHRHKRQLMADEPQSDHYKN

>20:53:38|GENSCAN_predicted_CDS_3|786_bp

Drosophila Gene Hit rescue sequence: pebble (rho1 GTPase exchange factor)

45 (AF136492)

Human Homologue BLASTX with pebble: KIAA0337 (AB002335)

71

Drosophila EST SD09146 (AI542703), SD01796 (AI530981)

Annotated *Drosophila* genome genomic segment AE003557

Annotated Drosophila genome Complete gene candidate CG8114 - pbl pebble rho1

GTPase exchange factor

Human homologue of Complete gene candidate 2224615 dbj BAA20795

(AB002335) KIAA0337 [Homo sapiens (3e-21) also mouse homologue 3e-95 42359 transforming protein (ect2) - mouse >gi|293332

(L11316) ect2 [Mus

musculus]

15 **Putative function**

A guanyl-nucleotide exchange factor involved in signal

transduction which is localised to the mitotic anaphase. pbl is required for the formation of the contractile ring and the initiation

of cytokinesis in Drosophila

20

5

10

Confirmation by RNAi Slightly reduced G1 and G2/M peaks indicating fewer cycling cells

72

Line ID 542/3

Category Mitotic defects in brain: cytokinesis defect

(very high polyploidy)

Reversion NR
Map Position 66A
Rescue ID 2A1E

Rescue Sequence

20 Genomic hit, Accession No. CSC:AC018042

Drosophila EST SD09146 (AI542703), SD01796 (AI530981)

rest of results same as line 293/9

73

Example 7 (Category 1)

Line ID 229/30

Category Mitotic defects in brain: cytokinesis defect. Meiotic defects in

testis: cytokinesis defects

(Mitotic higher level of condensation, polyploidy, Meiotic:

Ck05/07)

Reversion ? Map Position 91F

10

5

Rescue ID A7E

Rescue Sequence

Annotated *Drosophila* genome genomic segment AE003686

25 Annotated Drosophila genome Complete gene candidate CG6284 - novel protein

possible sir2 family of

transcriptional

regulators/telomeric silencing

30 Human homologue of Complete gene candidate gi7706710

0268A424791DE5BF

|ref|NP_057623.1| sir2-related protein type 6 [Homo sapiens]

(1.10E-74)

35

Putative function Putative transcriptional regulator

Confirmation by RNAi Complete loss of G1 and G2/M peaks indicating fewer

40 cycling cells

74

Line ID

1104/16

Category

Mitotic defects in brain, Cytokinesis defect (no overcondensation

of diploids, high polyploidy)

Reversion

R

Map Position

5

92A

Rescue ID

B5E

Rescue Sequence

20 Rescue ID

B₅P

Rescue Sequence

30 GCAAGTGCATTTATATTTGGAAATAATAAATGCTACAAT

other results same as 229/30

75

Example 8 (Category 1)

Line ID

343/5

Category

5

Mitotic defects in brain: cytokinesis defect

(very high polyploidy, chromosomes entangled?)

Reversion

NR

Map Position

75B

Rescue ID

C6E

10 Rescue Sequence

GCTGCCGCACACATTGGCCTCTCTCTCGCAGCTCCACATTCGAAGGTGGCTGA CCGAAATGTGGGTCACGACAATGGCGGGGTTCGTTGAACTGAACCACCGCCG CAGTCGCTGCCGTGCTCGCTCTCCTCTGCTGACGTCGTTAACGTTTTGGG 15 GCTTTCGGTTACGTAGCTCGTGTGCGAGCGAGAGGGGCTACTAGAGGGACTGC GACACACAGTTGTGCGTTTTTTTGGCCCCAAAAAATCACAATGGGCACAAA TTCATCGAACTGCCAGCGATTGACAAATTGCGATTTTCAATGCGGCAAAAATA TTTACTCAAGCAAATTGTTTGCACTTCGTTAATTAGGCGGGGAGTGCCGCCAA ATTGGGTCATATTGCAGAAGTATCCAAGAAGTTGGAGAAACAAGCTGCTTAA20 GTTACCCTTATATTAATTTTCAAATTTCTAAATAATCAA

Genomic hit, Accession No. CSC:AC015427

25

30

Associated ORF

Genscan ORF1 predicted sequences

MVCAMOEVAAVOHOOOOOLOLPOOOOOOOTTOOOHATTIVLLTGNGGGNL HIVATPQQHQPMHQLHHQHQHQHQHQQQAKSQQLKQQHSALVKLLESAPIKQQ OOTPKOIVYLOOOOOOPORKRLKNEAAIVOOOOOTPATLVKTTTTSNSNSNNTQT TNSISQQQQHQIVLQHQQPAAAATPKPCADLSAKNDSESGIDEDSPNSDEDCPN ANPAGTSLEDSSYEQYQCPWKKIRYARELKQRELEQQQTTGGSNAQQQVEAKPA AIPTSNIKOLHCDSPFSAOTHKEIANLLROOSOOOOVVATOOOOOOQOOHOHQQ QRRDSSDSNCSLMSNSSNSSAGNCCTCNAGDDQQLEEMDEAHDSGCDDELCEQH HQRLDSSQLNYLCQKFDEKLDTALSNSSANTGRNTPAVTANEDADGFFRRSIQQK

35 IQYRPCTKNQQCSILRINRNRCQYCRLKKCIAVGMSRDVLRLEQPKAGAKNKSCE **PSKNSTVNQINSKLELGNSNEMK**

>21:55:09|GENSCAN predicted CDS 1|1533_bp

40 atggtttgtgcaatgcaagaggttgctgccgtgcagcatcagcagcagcaacagcaactccagttgccccagcagcaacagcag cagcagcagacaacacagcagcaacatgcaacaactatagtgctgctgacgggcaatggcggcggtaatctgcacattgtcgcc acaccgcaacagcatcagccgatgcatcagctccaccatcagcatcagcatcagcatcagcatcagcaccagcagcaggccaagagcc aacagctgaagcaacaacactcggcgctggtcaagttgctggagtcggcgcccatcaagcagcaacagcagacgcccaagca aattgtttacctgcagcagcagcagcagcaaccgcaacgcaaaagactgaaaaacgaagcagcaatcgtacaacagcaacaac 45 cagcaacagcagcatcagattgtgttgcagcaccagcagcagcagcagcaacaccaaagccatgtgccgatctgagcg

76

Drosophila Gene Hit TBLASTN with ORF1: ecdysone-inducible gene E75B (X51549) and nuclear receptor superfamily protein (U01087) BLASTN with genomic sequence matches ecdysone inducible gene

Annotated *Drosophila* genome genomic segment AE003522

Annotated Drosophila genome Complete gene candidate CG8127 Eip75B ecdysone-

inducible gene E75B nuclear

receptor NR1D3

Human homologue of Complete gene candidate ORPHAN NUCLEAR

RECEPTOR NR1D1 (V-ERBA RELATED PROTEIN

EAR-1) (REV-ERBA-

ALPHA) Q15304 (9.40E-74)

Putative function Ligand-dependent nuclear receptor, putative transcription factor

Confirmation by RNAi Slightly reduced G1 and G2/M indicating fewer cycling

cells

5

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77

Line ID

448/23

Category

Mitotic defects in brain: cytokinesis defect

(very high polyploidy

Reversion

NR

5 Map Position

75B

Rescue ID

2G4E

Rescue Sequence

GCTGGTGGACGCTGCTTCATTCGCAAATTGCTCGTCGTTGGCAGCGGTTGTGC 10 AGAGCAAGAAAAGCGCGCGAAAAAACCAAGCAAAAAATTAATACAGCTGGAT CAAGCGAAAGAGATAGAGAGCAGCAGCAACAACAATGTTCAATAGCA AATGATATCGCATATTTTTGTTGGTGCCAGTGAAGTGAGATCAAAGTGAAGTG TGCAATGTTCCTTATTAGCAAATCGTAGAGCAACCAACAATCGAGAGTTCAAG TGTCATTCGAAGCCAAAAAGCAAAATCTCTAATTCAAATATGGTTTGTGCAA 15 TGCAAGAGGTTGCTGCTGTGCAGCATCAGCAGCAGCAACAGCAACTCCAGTT AACGATAGTGCTGACGGGCAATGGCGGCGGTAATCTGCACATTGTCGCCA CACCGCAACAGCATCAGCCGATGCATCAGCTCCACCATCAGCATCAGCATCAG CATCAGCACCAGCAGCAGCCAAGAGCCAACAGCTGAAGCAACACACTCGG CGCTGGTCAAGTTGCTGGAGTCGGCGCCCATCAAGCAGCAACAGCAGACGCC20 CAAGCAAATTGGTTACCTGCAGCAGCAGCAGCAGCAACCGCAACGCAAAAGA CTGAAAAACGAAGCACAATCGTACAACAGCAACAACAACACCTGCAACAC

Genomic hit, Accession No. CSC:AC015427 *Drosophila* EST GM03519 (A801874)

Other results same as line 343/5

78

Example 9 (Category 1)

Line ID

36/1

Category

5

15

25

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45

Meiotic defects in testis: cytokinesis defects

(Ck-04/06) `

Reversion
Map Position

R 79C

Rescue ID

A8B

10 Rescue Sequence

GAGTAAAGTAAACTACAGAGAAAAAACGCTTTACGGCGAGAGAACGCTTTAA
TTATACTTAATTTGTTGTTAATCAAACGCACAGAGCACACAACACACAGAAACAC
AAAACACCGCTTGGGAAAAATCTGTAGGTAGANGAAAGGAGCTCACGTTTT
CTGGTGCAGATCGAAATCGGTATCGGGTTTATTCGCTTTGCCGGATTGTTACTT
CACGTTTGTTAATTGCGTTTCTTTGTTTCTTATTCTCCTGCGCACACTTTGATTT
GCGTTTGCAACTCGCAATTCGCAATTGGCATTTGCTATGCGACAACTGCCGTT
ATTTCCGGTCCGTTTACTTTTCCAATGGCTTGCCTACACACCGCCAAACTTTGG
CTTGAACTTGGGATATTGGTTGCCCGAATTTCCTGANAAATTTTTCCTT

20 Genomic hit, Accession No. CSC:AC013886

Associated ORF

Genscan partial ORF1: >18:33:59|GENSCAN_predicted_peptide_1|99_aa CICFALLGLLIRRKLMVVFGSTSRKAQSLESRRAKNTSQKIGNQYPKFSQVCGKPS KSNDRNNGSCRIANANCELRVANANQSVRRRIRNKETQLTNVK

>18:33:59|GENSCAN predicted CDS 1|300 bp

tgtatctgcttcgcctgcttgggctactcattcggcgaaaattaatggtggtgttcggttctacgtcgcgcaaggcacagtctctaga gtctcgcagagctaagaatacatctcagaaaatcggcaaccaatatcccaagttcagccaagtttgcggcaagccatcgaaaagt aacgaccgaaataacggcagttgtcgcatagcaaatgccaattgcgaattgcgagttgcaaacgcaaatcaaagtgtgcgcagg agaataagaaacaaagaaacgcaattaacaaacgtgaagtaa

Drosophila Gene Hit rescue sequence and TBLASTN with ORF1: nucleic acid binding

protein (mub) (X99340)

35 Human Homologue BLASTX wit

BLASTX with nucleic acid binding protein: poly(rC)-binding

protein 2 (hnRNP-E1) (S42471)

Drosophila EST

several including LD32520 (AA951799 BLASTN matches nucleic

acid binding protein (X99340)

40 Annotated *Drosophila* genome genomic segment AE003596

Annotated Drosophila genome Complete gene candidate CG7437 - mub mushroom

bodies RNA binding protein

Human homologue of Complete gene candidate

4826886

ref|NP_005007.1|pPCBP2| poly(rC)-binding protein 2

79

>gi|542853|pir||S42471 (4e-75)

5 **Putative function** A putative RNA-binding protein specifically expressed in the CNS of Drosophila melanogaster

Confirmation by RNAi Only wild type profiles observed

80

Line ID

472/22

Category

Female sterile

(anaphase bridges, lagging chromosomes)

Reversion

?

5 Map Position

nd

Rescue ID

sau 5'spl

Rescue Sequence

10 GCACGATCNCTAAAGTCTNGCANAGCTAAAAATACATCTNAGAAAATCGGCA ACCAATATCCCAAGTTCAGCCAAGTTTGCGGTGTGTAGGCAAGCCATCGAAAA GTAACGACCGAAATAACGGCAGTTGTCGCATAGCAAATGCCAATTGCGAATT GCGAGTTGCAAACGCAAATCAAAGTGTGCGCAGGAGAATAAGAAACAAAGA AACGCAATTAACAAACGTGAAGTAACAATCCGGCAAAGCGAATAAACCCGAT

15 ACCGATTTCGATCGGTGCGGGCCTCTTCGNTATTACGCCAGNTGGCGAAAGGG GGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG ACGTTGTAAAACGACGCC

ANTGCCAAGCTCTGCTCTAAACGACGCATTTCGTACTCCAAAGTACGAAT TTTTTCCCTCAAGCTCTTATTTTCATTAAACAATGAACAGGACCTAACGCCNGT

20 AAC

Rescue ID

Sau 5'splac

Rescue sequence

25 GTTGTGATCNTCTTGGTNAATCNNNTTGGAAATTCCCCTAANGCTTCCGACAA
 TGACCCNGNCNTACNNAGCAAANAATNGNAGNACNNGCNGNTGGNCGTANT
 ANCAANAACAGGCCCGCACCGATCGAAATNGGNATCGGNTTTATTCGCTTTGC
 CGGATTGTTACTTCACGTTNGTTAATTGCGTTTCTTTGTTTCTTATTCTCCTGCG
 CACACTTTGATTGCGTTTGCAACTCGCAATTCGCAATTGGCATTTGCTATGCGA
 30 CAACTGCCGTTATTTCGGTCGTTACTTTTCGATGGCTTGCCTACACACCGCAAA
 CTTGGCTGAACTTGGGATATTCGTTGCCGATTTTCTGAGATGTATTCTTAGCTC

Other results same as line 36/1

TGCGAGACTCTAGAGACTGTGC

81

Example 10 (Category 1)

Line ID 459/2

Category Mitotic defects in brain: cytokinesis defect. Meiotic defects in

testis: cytokinesis defects:

(mitotic: high polyploidy, no diploids, higher mitotic index,

meiotic: Ck-01/05)

Reversion NR **Map Position** 66B1-6

10 Rescue ID

5

2D5P

Rescue Sequence

TCCAGAACAG

25

30

Annotated Drosophila genome genomic segment AE003557

Annotated Drosophila genome Complete gene candidate CG8038 - novel gene ribonuclease P homology CG7892 nmo - protein serine/threonine kinase involved in eye morphogenesis

35 Human homologue of Complete gene candidate

CG8038- 5e-24 4309676 gb|AAD00893| (AF001176) ribonuclease P protein subunit p29 [Homo sapiens]

40 CG7892- protein kinase mitogen-activated 7 (MAP

kinase)' gi:4506093 and gi7706445 D919050533B3C33A

45 |ref|NP_057315.1| nemo-like

82

kinase [Homo sapiens] (3.30E-174)

5 **Putative function** CG8038: tRNA processing enzyme Ribonuclease P protein subunit CG7892: a protein serine/threonine kinase involved in cell cycle, possibly targeted to cytoskeleton

Confirmation by RNAi Both showed a marked increase in G1 peak indicating arrest in G1

WO 01/72774

83

PCT/GB01/01297

Example 11 (Category 1)

Line ID 623/8

5 Category Meiotic defects in testis: cytokinesis defects

Reversion

Map Position 37E1-3

Rescue ID 2E2E

10 Rescue Sequence

25 CAGT

Annotated *Drosophila* genome genomic segment AE003662

Annotated *Drosophila* genome Complete gene candidate CG17559 dnt - doughnut protein tyrosine kinase

30

35

15

Human homologue of Complete gene candidate

Homo sapiens RYKreceptor tyrosine kinase GDB:21773

Putative function growth factor transmembrane receptor protein tyrosine kinase involved in cell growth and maintenance

Confirmation by RNAi Only wild type profiles observed

84

Example 12 (Category 1)

Line ID 629/14

Category Meiotic defects in testis: cytokinesis defects

(Ck-06/09)

Reversion NR **Map Position** 64D

Rescue ID 2A9X

10 Rescue Sequence 1

5

Rescue ID 2A9E

Rescue Sequence 2

CTCCCGTCGTTTTGAGATCAGCTGCTCTCGCAACAACAACAACAACTATAACTGTA
GTTACCGTCTCTTTTTGCATCGTTCGTTTTTCGTTTGTCGCCAAGTGATTGTGT
GTGTGCGTAAGCTTAAAGCTGACTAACAAAACGAAACAAGAAAAAAATATAAA
TTATAGGAAAATTGTTAAATTATAACCAGAAAGAGAGCGGCACTTACGTGTGT
TATTGTGTGCGTGTGCTTTAAAAAGATATAAAAATAGCAATAGAAAGTTATTA
AAGCGTTGGCAAAAAAGTCCAACGAACAGCGAGAGGAAGCGGAGAACGAAA
TAGTTAAAGCCAAAGTCGCTGCCGACGTCGCACTTGAAAAACGTCGCAAAAGTT
TGTAAACACACCAGTGTGTGTTCGTGTGTTTTTTGCCGGCGTGCCAGTGTGCG
TGCGCCTAGAAAAGAGTAAAGAAGCAGAAGAAAAAGGAAGAAGAAGCCGAAGAAG
CAGCAAAAGAAGCCGACAGCAAAAAGTAAATAAAATCAAATGCCCCCTGGCA
 GAATAATATTAAATTAAGACACATACTCAAATTAATAAC

Genomic hit, Accession No. CSC:AC015076

Drosophila EST LP08767 (AI295205)

40

Annotated *Drosophila* genome genomic segment AE003567

Annotated *Drosophila* genome Complete gene candidate CG10668 - novel with homology to ssDNA/RNA

nomology to SSDNA/KNA

binding proteins

45 Human homologue of Complete gene candidate CG10668 - 3e-12 4506449

85

ref|NP_002889.1|pRBMS2| RNA binding motif, single stranded interacting protein 2 >gi|1082

5

Putative function Possible single stranded DNA/RNA binding protein

Confirmation by RNAi Slightly increased G1 and reduced G2/M indicating G1

10 arrest

86

Example 13 (Category 1)

Line ID 653/12

Category Meiotic defects in testis: segregation defects, cytokinesis defect

(Ck-07/35)

Reversion NR **Map Position** 75B

Rescue ID I5E

10 Rescue Sequence

5

15

20

Genomic hit, Accession No. CSC:AC014071

25 Associated ORF

Genscan ORF1 predicted sequences >16:36:33|GENSCAN_predicted_peptide_2|477_aa MLILMRPSIKLAANQNAIKAPNGPKNFLDKVLVVRCWLSVCLLENGHIAVTASGS NNNNNSNNINLNLKANYQMSATSIRDSFATILLDAQNRVQNATVAAKNFMLPLR LRSDTSGDTSNNNENNSRRARQAYNCGVNWLTTHRPKRRRQVHPPLGSTPSCNN

- 30 NSSKISRNSSSSSNNIASATATRIFLGTSAILAIDFDNTRVPGYYQPTGEWIWVSKS MIKQLFAVAATADDVAAAAASRGNALTFLPGKEKGPRKKAEGCGMEWSGVEWS GGDVMCVLSSVATVDDDDHHGGGHFDGLLGTPSALIRLNCLINPKKMRMDFEVE VAWQIARAADLRLISMHLNVPYEMKTMKTMESVIDGGSLYQPTALFGSLFCLVY SSAADVLLLLANCKSLAHGVDVDCDSDASRGSDCDVGHFSPSFRCRFQLSLVAQS
- 35 ARHANALKSQVTTATSSSSNNSDSLANKQTNQHIFVYQLSA

CCTGCACACGCATCCCCATAAAGAACGACCTTGAGCT

>16:36:33|GENSCAN predicted CDS 2|1434 bp

87

10 Drosophila Gene Hit rescue sequence, ORF1 and genomic sequence: Canton S E78B

nuclear receptor superfamily protein (U01088)

Drosophila EST LP11082 (AI296953 similar by BLASTN to U01088)

Annotated Drosophila genome genomic segment AE003593

5

20

25

15 Annotated Drosophila genome Complete gene candidate CG18023 - Eip78C

Ecdysone-induced protein 78C nuclear receptor NR1E1

Human homologue of Complete gene candidate CG18023- 4e-32 119100

P20393 EAR1_HUMAN V-ERBA RELATED PROTEIN

EAR-1

>gi|1082832|pir||A32608

Putative function ligand-dependent nuclear receptor, putative transcription factor

Confirmation by RNAi Not done due to failure of PCR procedure

88

Example 14 (Category 1)

Line ID 876/2

Category Meiotic defects in testis: cytokinesis defects

5 Reversion ?
Map Position 73A

Rescue ID 2H1E

Rescue Sequence

Genomic hit, Accession No. AC005633

Drosophila Gene Hit rescue sequence: argos (M91381

25

35

Annotated *Drosophila* genome genomic segment AE003527

Annotated *Drosophila* genome Complete gene candidate CG10162 – Egf2 translation

facto

30 Human homologue of Complete gene candidate CG10162 - 4e-11 181969

(M19997) elongation factor 2

[Homo sapiens]

Putative function Translation elongation factor

Confirmation by RNAi Not done due to failure of PCR procedure

CATEGORY 2: FAILURE TO ENTER M-PHASE

Example 15 (Category 2)

Line ID 1216/12

5 Category Meiotic defects in testis: no division

(no meiosis)

Reversion NR **Map Position** 82F1-2

10 **Rescue ID** 2I5X-1

Rescue Sequence 1

15

20

Rescue ID 2I5E-1

25 Rescue Sequence 2

- 30 ATAGAAATGTCGACGCACCCTTTTCTTTTTCTCGCAAAGAACGAGGAAATGGA GAAGCGCAAAACCACATCCCGCTTAAAGAGTCCCTTTCCCCCGCTGGAAGTGG AAGGAAAGGCAGCTTAAAGAGGAGCGGGTGGCTTCCAGTATGTGGAAAACAA AGCAGACGCCATTGGAATGCCGTCGTTTTTTGTTGTTGCTAAGCCGGACATGG CAATTGTTGCTTTTTTTCGAGAGGGGGTGGTGAAACTCATAAATATCAGCT
- 35 ATGGCGAGGGGGGGGGCAGTCTTTGTCTGACGTACCGTACTTTTAATTTCTT GTCGCCCGGTTTAATCCAATTTATCCAGCTTTGAATTTCGCGG

Genomic hit, Accession No. AC007532

40 Annotated *Drosophila* genome genomic segment AE003603

Annotated *Drosophila* genome Complete gene candidate CG1116 - novel

Human homologue of Complete gene candidate 2495728 HYPOTHETICAL PROTEIN KIAA0258(aa)

90

Putative function No homologies which indicate function

Confirmation by RNAi Slight loss of G1 peak

91

Example 16 (Category 2)

Line ID 1344/15

Category Mitotic defects in brain: no mitosis

5 Reversion NR Map Position 83C

Rescue ID 2F6E

Rescue Sequence

10 AGCGGGAGTGAGCCGAAAGAGAGTAATTTTGGCCGTCACCAAAAAAAGTGGCT
GCATAGTGCCAAACCAATGTATGGCCGTTACGCATCTTGTTATTCTAGTGTCTT
TGGCTGTAATCAGTTTGCAGTGACAGAGGAGTTCAGTTTCAGTTGACTCGGCT
TGGTTCAGGGTTTCTGATTGCCGTCCTCTTCTCCCTCTTCGCCTACAAGTCCGC
TGTTCGGCACCGTGACGTCACCTAGACTTACACCCCTAATCAAAGATCCACTA
15 GTTTAGATTTCCTGCATCAACGCCATATTAACTTTATAAGCAGTCGTTATATCT
CAAGTAGGCAAAAAAGTGTAATAGATATGTATCTAAATTGTCGTACATTCTAT
TTATTAAAATTCGTTTTTTACATCCAACAGGTGTTATTTTTGAAGTCTTAGATAA
CAAACAATATTCGAATTATGTGGTAGAATACTTAGCAATATCAAGACAACAT

20 AATGCAACATCTGGTCCGAGCTATCCAGGCAATCACATTTTTGAAGTTCCCCC GGTTATCACACATATATCGATCATACCCCGAAATGTGTAACACAGATACAGCT CACCATCCCTCTGATAAGATCTTATCAAGTTCGGGCTTGCTCGCTATCGTGAAT TGGCTGAAGGGTCCGCGATAATTGCATTGGGCATGCCATTGGTAATCACAAT TGGCTGATAATGCTGCTGCAATTCCACGGGTATGAA

25 TTCATCAATTGGTTA

Annotated *Drosophila* genome genomic segment AE003602

Annotated Drosophila genome Complete gene candidate CG1347 - novel protein with

myosin homology

30 Human homologue of Complete gene candidate 1503990 |dbj|BAA13194|

(D86958) KIAA0203 similar

to mouse CC1.(aa)

Putative function similar to coiled coil protein with ubiquitin like domain

Confirmation by RNAi Marked reduction of G1 and G2/M indicating fewer cycling cells

40

92

Example 17 (Category 2)

Line ID

703/16

Category

Meiotic defects in testis: segregation defects, meiotic failure

(Mf-07/75)

5 Reversion

R

Map Position

83B

Rescue ID

2E7E

Rescue Sequence

10 AAGCAGCCCAACAGCTACGCAAAAAGTTACTTATATTCGCAGCAAAACAGAT TTTTTTGTTTTAATCGTAAGTATAGGAGTGAAAAATAGCGCTAGAGTAGACCT AAGTACACAGAAAGACAAATAGGGCGAGTAAAATCGCGGTCCTGGTCATTTC TCTGGCCTTGACCAATCCTTTGTCTGCGCTTTCGTTGGAAAAGGGGTTATGTAC GAACTGCGTGCGTACCTAAGGCCAGATTAGTCATCGGGCAGTCATATATTCAT

15 GCAAAAATCATTTGGTGGCCGTCGGCCTTTGTTCGACTGTACCTTGCTCATTA
TTTAATAAGCGCGACAGCAATATACACACTTTGAACCCCCATCCCACATTTTTT
CTCACCGTTTCCCCCTAATTTTCGTTTTCCCTGTGCCCATCATTCCGCTTTCGCC
ATGTCAGTGTATCGCTTCAAAATGGCGCCGAACCACATGTCTTCGTTCTCGGC
TCGTCCGCTTCGTTCGTGCGCTCGTGTCTCATTCGCTCTCCGAATTTCG

20 TTTAACAAAGTGGTGCGAGCAGAGGGGCCGCTGGATTCGAGGCAAACAACAC ATATACCTA

Genomic hit, Accession No. CSC:AC013960

25 *Drosophila* EST several including LD15903 (AA440858), GH20091 (AI389018).

Annotated *Drosophila* genome genomic segment AE003602 Annotated *Drosophila* genome Complete gene candidate CG2922 – novel

30 Human homologue of Complete gene candidate 286001 dbj[BAA02795] (D13630)

KIAA0005 [Homo sapiens] also NP_054757.1| HSPC028 protein

[Homo sapiens] e-179

35 **Putative function** Weakly similar to a region of human and murine

EIF4G2 translation initiation factors; may act as a

translation initiation factor

Confirmation by RNAi Only wild type profiles observed

93

Example 18 (Category 2)

Line ID

5

15

741/3

Category

Meiotic defects in testis: segregation defects, meiotic failure

(Mf-05/31)

Reversion

NR

Map Position

88D

Rescue ID

H₆E

10 Rescue Sequence

GCCTGGAGCCACCTCTAGAGCCACGGCCAAAAAATTGTGTGCCAAAAAATCG TATGGCGTTACGCATCTTGTTATTCTAGTGTCTTTGGTTCTACAAATCTGGCCA ATGGGATGGACGGATTTTGGGGCTTTTGCGCCCCACATATGTNTCTTACAACC CACTCGGCCGGCAAGTGGGTGTCAATTACGGACATCGGCAATCCGAAGACC GGAGACCCAGAGCCCCAGGGCCCCATTCGATTCGATTTCGAGTT GCGTGGGCCGATCTCACATTAGTCACATCGAAGGAATGAAATAAAAAGAAAA AACATGACGGCCGAAAAGAACTTATCCATCTTCAAAGCTCTCAGAAAATACA AAAAACTAAAAAACTTTTGACTCTTCGTCTTTCACATTTCGAAATCACAAAAT GTCTGCCATAAATTCCAAAGTGAACAATTGAAATAAATTTTTGCGCCATGAAC

20 ACGCCGACTG

> Annotated Drosophila genome genomic segment AE003705 Annotated Drosophila genome Complete gene candidate CG12600 - novel protein

25 Human homologue of Complete gene candidate

CG12600- 5e-27 4240227 dbj|BAA74892.1| (AB020676) KIAA0869 protein [Homo sapiens]

30 **Putative function** putative cytoskeletal structural protein

Confirmation by RNAi Reduction of G1 and G2/M peaks indicating fewer cycling cells

94

Example 19 (Category 2)

Line ID 773/1

Category Meiotic defects in testis: cytokinesis defects, meiotic failure

(Mf-02/15)

Reversion R? Map Position 83F

Rescue ID 2D9P

10 Rescue Sequence

5

25

30

35

- 20 GGGTTAATCATTTTCTTGCTCCATCTGCTTTTCCCAACTGTATCCAAGTACAAC TACAGCATTATCCTCAACTG

Annotated *Drosophila* genome genomic segment AE003675

Annotated Drosophila genome Complete gene candidate CG10272 - novel protein

Human homologue of Complete gene candidate CG10272 - 2995577

AC004490 (AC004490)

R29381_1(aa) protein includes HMG-I and HMG-Y DNAbinding domain (A+T-hook) found in HMG non-histone components in chromatin

Putative function Chromosomal protein

Confirmation by RNAi Loss of G1 peak indicating arrest in G2/M

95

CATEGORY 3: METAPHASE ARREST

Example 20 (Category 3)

5

Line ID 1067/13

Category Mitotic defects in brain: prometaphase arrest

(overcondensation, polyploidy, scattered chromosomes with

bipolar spindle)

10 Reversion NR

Map Position 69C4-10

Rescue ID 2F8E

Rescue Sequence

15 GTTTGGGCACAGGGTTGTATTTCATTTATTTTTGGGGGGAGTCGATACGCTCTC
TTGGCGTGGTCGAACGGTCACACTGGCCGAGAGATAACGGAAAATGTTTCAA
AGGTAAGTAAAAGATTATAAACGTATTAAGCTTAATACTATAATTAGCTTACTA
TTCCAAGTATGTATAATTATATACACGTTTAAAAAGGCATAACGTTAAGTGTAAC
CAAATTATTAAAATGACATATATTTAAGCCTCTTTATATATGTAAAATGTTAA

20 TTAATATTAAATCACATATATTTAAGCCTCTTTATATATGTAAATATTTTAA TTTTATTAAAATAAATTATATATTGTTTTGTAATATGATCGAGGGCTGCCACCT TGTGATAAATGCTTACCAACACTTTTAGGTACGCCGTTTAGTGTACGTAAGTTG CGTACCTAGATATCCAGCGAAATCAAAACATTGAGTAAATCGTGGAAAATGG ATGAAAATAGCTTAATCTACGGACTCGAACTGCAGGCGCGGGCTTTAACACCT

25 CAGTACGGAGAGCAACGATGTGTGCTTCTTCATAGCCACCAACTCCTTGAA GCCCACCAATCAGGTTCACTTAATCCAGTACGAAGA

Genomic hit, Accession No. CSC:AC020333

30 Associated ORF

Genscan: ORF1 predicted sequences: >16:51:11|GENSCAN_predicted_peptide_2|178_aa MAQNISPEQSGGAGGGSKHSDDSMPVKDNHAVSKRLHKELMNLMMANERGIS AFPDGENIFKWVGTIAGPRNTVYSGQTYRLSLDFPNSYPYAAPVVKFLTSCFHPNV DLQGAICLDILKDKWSALYDVRTILLSIQSLLGEPNNESPLNAQAAMMWNDQKEY

35 KKYLDAFYEKHKDT

>16:51:11|GENSCAN predicted CDS 2|537 bp

96

Drosophila Gene Hit TBLASTX with ORF1: poor homology to several sequences

including homolog of RAD6 (DHR6) (M63792), bendless

(L20126) and Ubc D1 mRNA for ubiquitin-conjugating enzyme (

X62575).

Human Homologue TBLASTX with ORF1: ubiquitin carrier protein E2-C (UBCH10)

(NM 007019.1) and ubiquitin-conjugating enzyme E2B (RAD6

homolog) (NM 003337.1).

Annotated *Drosophila* genome genomic segment AE003541

10 Annotated Drosophila genome Complete gene candidate CG10682 – vihar ubiquitin-

conjugating enzyme

Human homologue of Complete gene candidate gi5902146

0B6F58A1F0665D9A

|ref|NP 008950.1| ubiquitin

carrier protein E2-C [Homo

sapiens] (2.50E-50)

20 Putative function Cyclin specific ubiquitin conjugating enzyme

Confirmation by RNAi Complete loss of G1 and G2/M peaks indicating fewer

cycling cells. Immunostaining shows metaphase arrest with

condensed chromosomes

15

97

Line ID

1105/1

Category

Male sterile, Female sterile, Mitotic defects in brain: prometaphase

(Overcondensation, polyploidy, fewer anaphases, high mitotic

index, scattered chromosomes with bipolar spindle)

Reversion

5

15

Map Position 69C

Rescue ID

A₅B

10 Rescue Sequence

GTACATATAATCACAATTGAGAATCGAAAACCCGACCGCCACGAAGCGCGCT AAATTACACGCACATACTGAAAGCCAAACAGCGGATAGCACTAGCATCCTAC ATATAGACGTAGATATAGTCATGGCGCAGAATATCAGCCCCGAGCAAA GTGGTGGAGCAGCGGCGCGCGCAGCAGCACAGCGATGACTCCATGCCCGT GAAAGACAATCACGCCGTGGAGCAAAAGGTGAGTATCACATGGTGCAGCCTA GGGAACTGATGAACCTGAATGAATGGGCCCACCGAAAAAAGGGG

Rescue ID A5E

20 Rescue Sequence 2

ATATGTACTGTATAGTGGAAATTTAGTTTGATCGGTCGGAATACGCGTCTGTT ${\tt GCTTTTCAGATATTTTTTTTCACTTTTGTGTGAAAACAAAATGGAAGGAGA}$ GAGTCGATACGCTCTCTTGGCGTGGTCGAACGGTCACACTGGCCGAGAGATAA

- 25 CGGAAAATGTTTCAAAGGTAAGTAAAGATTATAAACGTATTAAGCTTAATACT ATAATTAGCTTACTATTCCAAGTATGTTATAATTATTACACGTTTTAAAAGGCA TAACCGTTAAGTTGTTAACCCAAATTATCAATGGATTTTGAATACCAATATT ATTTATTTTATATTTTGAGCTTAATATATATAAATCCACATATATTTAACCCCCCT TTATATATGTTAAATATTTTAATTTATTAAAATAAATTATATATTGTTTGGTTA
- 30 AAA

Genomic hit, Accession No. AC007328 69B-69C

Associated ORF

35 Genscan: ORF1 predicted sequences

>/tmp/aaaaanjda|GENSCAN predicted peptide 1|357 aa

MGKKAKHKKKGKGPEKTAMKADKKQAARQKKMLEKLGEANIADIIQLLEAKEG KIEAISESVCPPPTPRSNFTLVCHPEKEELIMFGGELYTGTKTTVYNDLFFYNTKTV EWROLKSPSGPTPRSGHOMVAVASNGGELWFPNFACISRNOSWFVFHNCRLKAA

- SREKVLLNFNGTVLHPANNIIVHVKLFKKANGFKPWLLDVKLDACRFVRTNFHPF 40 VRIIFDLFKDFSTINHTCPYVVLRSMRYIVRRSPRLVHPIVDVPAIGHTRPRRKAAV RGIGCAHRCPLIRMATPCRTNVVMMTLMRGSVRSRVMAICCYRRPAIAIARRRHP TAIAHSQEVAERLGGLLYPDIQRTNP
- 45 >/tmp/aaaaanjda|GENSCAN predicted CDS 1|1074 bp atgggcaaaaaaggccaaacacaagaagaagggcaaagggcccgagaaaacggccatgaaagcggacaaaaagcaggcgg cgcggcaaaagaaaatgctggaaaaactgggagaagcaaatatagctgatatcatccaattgctggaggccaaggagggcaag attgaagccatcagtgaatccgtttgcccgccaccaactccacgatccaatttcaccttagtttgccatccggaaaaggaggagctc

98

Drosophila EST several ESTs including LD04777 (AA201675)

All other entries as for 1067/13.

5

99

Example 21 (Category 3)

Line ID

1407/13

Category

Mitotic defects in brain:

5

(weak overcondensation, metaphase with bipolar spindle)

Reversion

NR

Map Position

92B1-3

Rescue ID

2D3P

10 Rescue Sequence 1

Rescue ID 2D3E

Rescue Sequence 2

TNCGTGATTATCAGCGTTAATTGTACAATATTATGATTTATTCGAGCTGTAAAT
 CTTCACAGCAAGCACAAACTGTAATTATACCACTTAGAATTCCGCGGAATTAA
 TTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCAT
 GATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGGAAATGTGCGCG
 GACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA

 GACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAG
 TATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTTGCGGCATTTTGCCTTCCT
 GTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTG
 GGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAG

35 *Drosophila* **EST** LD05707 (AA246767)

Annotated Drosophila genome genomic segment

AE003727

Annotated Drosophila genome Complete gene candidate CG7444 - very short ORF

with EF hand homology

Human homologue of Complete gene candidate

none

45 Putative function

40

Possible calcium binding protein

100

Confirmation by RNAi Slight loss of G1 peak

101

Example 22 (Category 3)

Line ID

1439/7

Category

Mitotic defects in brain: prometaphase arrest.

5

(overcondensation, polyploid, no anaphases, scattered

chromosomes with bipolar spindles)

Reversion

Map Position

96F10-14

10 Rescue ID G3X

Rescue Sequence

GTCGGATGTAGAAGACGTGCCCGAAACCCAGTTAGAAATCGATGTCAGCGAT GGCGCCGGACTGGAGGATGAGGATGACGATATGGAACAGATTACAGCTC AGAAGGTAAGGTAAATCGTAACAGAGCTTTTTAATACGCAAGTAATCACATTC 15 TGATATCCCTAGGTTCTGGAAATCATAGAAACCGCGTGGATAAATGAAATGTG TGCGCCGGAGATCCTGCCCAGCCAGACGGACATGCTGGAGCTGATGGTCTCCC AGGTGGCCCATATGGAGGAGCAGATGCGCGATCTGGACAAGAACGATTTCCG AGCGGTGGTGCACTCCATGGAACTGGAGAGGGTGCGCTACATAATGGCCAGT TATCTGCGTTGCCGCCTGCAGAAGATCGAAACCTTCACGCAGCACATCCTCAA CCAGGAGGAGACCGTGAGCCGGATGACAAACGTCTGTCTCCCGAGGAGACT 20 AAGTTCGCCCAGGAGTTTGCCAGTAAT

Genomic hit, Accession No. AC007825

25 Annotated Drosophila genome genomic segment AE003754 Annotated Drosophila genome Complete gene candidate CG14549 - novel

Human homologue of Complete gene candidate

none

30

Putative function no homologies which indicate function

Only wild type profile observed Confirmation by RNAi

102

Example 23 (Category 3)

Line ID 1466/4

Category Mitotic defects in brain: metaphase arrest.

(overcondensation, no polyploidy, fewer anaphases, metaphase

with bipolar spindle)

Reversion NR **Map Position** 72F

Rescue ID E5E

10 Rescue Sequence 1

5

GGCTGGATGCGATTCGCTTTCGGATTCGGATGGATTCAGCCGCTGTCTCGACA CCGCCGCAACCGCTCTCGGGAGTTTGAAAATTTGAAATGAGCGGATTCGCGTT GCGAAGGCGAGCTAGCGTTGCAGGCAGTGTGGCCAGATGCCGCGTGCGAACG TATTCTCGAATGCAATCGGCCGAGTGCAGATGCACTAAAAATAACCCACTTCC

- 15 AGTGACTGGAAATTAAGATCAAGGNAATAGATTTTATAAAAACTTATATGAGT AAAAATTTTAAAATTGTGGAGTCAACCTAAATTATAAGCAACTAATTTATAAC ACAAGTAAAGAATGATATTAAGTAACTTTTTAAATAATATTCCATTATGCTTA CGCTCAATTTATGAACAAATGTTTTCTCGATCCTTAGGTAAAGTTTCGAGTTTC GCGACTANATTTATTAAAAATTAAGAACATCTCCATTTATGTACACATTTAAAG
- 20 ATTTATGAGCGGTAATATTAGCTGGTTGAC

Rescue ID E5P

Rescue Sequence 2

30 AAACGCCCGCAGTGGCGGCGGCGGAAAAATCAGAGGAGCCGGAAAAGTCAG CGGCCCGCCAGCGGACTCAGCGGCCGCTCCAGCTGCCCCCCCGCAGTGGA GAAGGCTGAGGATGCCGATGGCGAAAAAAAGGACGGCGAGGCCGGAAAGCA GGACAAGCAGCAGGATGGC

35 Genomic hit, Accession No. CSC:AC020154

Associated ORF

Genscan ORF: ORF2 predicted sequences

>21:06:03|GENSCAN predicted peptide 5|415 aa

- 40 MASEVAQIPAEETPÄVAAAEKSEEPEKSAAPPADSAAAPAAAPAVEKAEDADGE KKDGEAGKQDKQQDGEEPKKDEAVAAPVATKSEAPPAQKFNVHKTNFEKDIIYL YQFSRTPLLPSLSPYCLKVETWLRLVGLKYENVDHKMRFRSKKGQLPFIELNGEEI ADSAIIIKELSSKYEKYLDSGLTAEQRNVSYATIAMLENHLIWIIFYWRAKYPDNV LKGYKVNLQHALGLRLPNSILNFFFKITFGRKGTKKLKAHGIGVHSAEEIEEFGKD
- 45 DLKVLSEMLDCKPFFFGDEPTTLDVVAFAVLSQLHYLSKDIAYPLRDYMTEKCPN LIGHVSRMKDKCFPDWDEICTKLDLNAHIPKPEPETKEGKEGGEQEKSNEQEGTE

103

GDKIEKELEKDKSNEKESTEENKEKEETK

>21:06:03|GENSCAN predicted CDS 5|1248 bp

atggcaagcgaagtggcccaaatacccgccgaggaaacgcccgcagtggcggcgggaaaaatcagaggagccggaaaa 5 gtcagcggcccggcagcagcggccgctccagctgcgcccccgcagtggagaaggctgaggatgccgatggcga gaagaaggacggcgaggccggaaagcaggacaagcaggatggcgaggagcccaaaaaaggacgaggcggtggcagc gtaccagttctcgcgcaccccactgctgccctccctgtcgccctactgcctgaaggtggagacctggctgcgtcttgtgggcctga aatacgagaatgtcgatcataagatgcgtttccgctccaagaagggtcagctgccgttcatcgagctgaatggggaggaaatcgc 10 cgattcggccatcatcatcaaggaactgtcgtccaaatacgagaagtacctggactcgggactcaccgccgagcaaaggaatgt ctcgtacgccacgattgccatgctggagaaccatctcatctggatcatcttctactggcgcgccaagtatccggacaatgtgctcaa gggctacaaggtcaacttgcagcacgccttgggctgcccaactcgattctgaacttettetttaagatcacetttggtcgc aagggcacgaagaagctgaaggcgcacggcatcggtgtccacagcgccgaggagatcgaggagttcggcaaggacgacctg aaggtgctcagcgagatgctcgactgcaagcctttcttcttcggcgacgagcccaccaccctggatgtggtggccttcgctgtcct 15 ctcgcagctccactatctgtccaaggacattgcgtatccgctgcgcgactacatgaccgagaagtgccccaacttgattggccacg tatetegeatgaaggacaagtgetteecegactgggaegagatetgeaegaagetggaeeteaatgegeacatteecaageeag agcccgagaccaaggagggcaaggagggggggagaagcaaggagaaatcaaacgaacaggagggcactgagggcgacaagat cgagaaggagttggagaaggacaagtcaaacgagaaggagtcgaccgaggagaacaaaggagaaggaaacaaagtaa

20 Drosophila Gene Hit rescue sequence and TBLASTN with ORF2: failed axon

connections (U21685)

Human Homologue BLASTX with fax: Metaxin 1 and 2 (Q13505 and AF053551) **Drosophila EST** several including LD31362 (AA951078 similar by BLASTN to

U21685 failed axon connections)

25

Annotated *Drosophila* genome genomic segment AE003527

Annotated *Drosophila* genome Complete gene candidate CG4609 – fax failed axon connectionsconnections

30 Human homologue of Complete gene candidate 4505281

ref|NP_002446.1|pMTX| metaxin>gi|3024205|sp|Q135 05|MTXN_HUMAN

METAXIN (4e-06)

35

Putative function Drosophila fax is a dominant genetic enhancer of the Abl mutant,

developmentally expressed in axons of the CNS

40 Confirmation by RNAi Weak reduction of G1 and G2/M peaks indicating fewer

cycling cells

104

Line ID

262/20

Category

Mitotic defects in brain: metaphase arrest.

(overcondensation, polyploidy, aneuploidy, few anaphases, high

mitotic index, metaphase with bent bipolar spindle)

5 Reversion NR

Map Position

72F

Rescue ID

G6E

Rescue Sequence

AGCTGCACGATAGGATATCTTGGCTGGATGCGATTCGCTTTCGGATTCGGATG10 GATTCAGGAGCCGCTGTCTCGACACCGCCGCAACCGCTCTCGGGAGTTTGAAA GTGGCCAGATGCCGCGTGCGAACGTATTCTCGAATGCAATCGGCCGAGTGCA GATGCACTAAAAATAACCCACTTCCAGTGACTGGAAATTAAGATCAAGGAAT

15 AGATTTTATAAAAACTTATATGAGTAAAAATTTTAAAAATTGTGGAGTCAACCT AAATTATAAGCAACTAATTTATAACACAAGTAAAGAATGATATTAAGTAACTT TTTAAATAATATTCCATTATGCTTACGCTCAATTTATGAACAAATGTTTTCTCG ATCCTTAGGTAAAGTTTCGAGTTTCGCGACTAGATTTATTAAAATTAAGAACA

TCTCCATTTATGTTCCC

20

Drosophila EST

several including LD28084 (AA949260)

All other results as for line 1466/4

105

Line ID

262/22

Category

Mitotic defects in brain: metaphase arrest.

(overcondensation, polyploidy, few anaphases, high mitotic index,

metaphase with bent bipolar spindle)

5 Reversion

NR

Map Position

72F

Rescue ID

F1E

Rescue Sequence 1

15 AGATTTTATAAAAACTTATATGAGTAAAAATTTTAAAAATTGTGGAGTCAACCT AAATTATAAGCAACTAATTTATAACACAAGTAAAGAATGATATTAAGTAACTT TTTAAATAATATTCCATTATGCTTACGCTCAATTTATGAACAAATGTTTTCTCG ATCCTTAGGTAAAGTTTCGAGTTTCGCGACTAGATTTATTAAAATTAAGAACA TCTCCATTTATG

20

Rescue ID F1P

Rescue Sequence 2

CGAA

Drosophila EST several including LD28084 (AA949260), LD38479 (AI518768)

35 Other results as for line 1466/4

106

Line ID 262/3

Category Mitotic defects in brain: Metaphase arrest

(overcondensation, polyploidy, aneuploidy, no anaphases, high

mitotic index, metaphase with bipolar spindle)

5 Reversion NR **Map Position** 72F

> **Rescue ID** H₃E

Rescue Sequence

AGCTGCACGATAGGATATCTTGGCTGGATGCGATTCGCTTTCGGATTCGGATG 10 GATTCAGGAGCCGCTGTCTCGACACCGCCGCAACCGCTCTCGGGAGTTTGAAA TGGCCAGATGCCGCGTGCGAACGTATTCTCGAATGCAATCGGCCGAGTGCAG ATGCACTAAAAATAACCCACTTCCAGTGACTGGAAATTAAGATCAAGGAATA

GATTTTATAAAAACTTATATGAGTAAAAATTTTAAAATTGTGGAGTCAACCTA 15 AATTATAAGCAACTAATTTATAACACAAGTAAAGAATGATATTAAGTAACTTT TTAAATAATATTCCATTATGCTTACGCTCAATTTATGAACAAATGTTTTCTCGA TCCTTAGGTTAAGTTTCGAGTTTCGCGACTAGATTTATTAAAATTAAGAACATC **TCCCTTTATGTTC**

20

Other results as for line 1466/4

107

Example 24 (Category 3)

Line ID

5

238/20

Category

Mitotic defects in brain: metaphase arrest

(overcondensation, metaphase with bipolar spindle

Reversion

NR

Map Position

75E1-3

Rescue ID

D7E

10 Rescue Sequence

TTCAGTCGCGCATTTCACCGTTTCCGAATCGGACGAACCGGGCGTGATTGCTC
TCCTGCTGCTTTCGAGATCGGAGTCCCGATAAGGATATAACTACAACCTAAAG
AGGAATCCAAGCCTCCTCCTGCCGCTAGTTTCGAAAAGTAAATAGAGTACTTG
TTATCAACTGGGGAAGCGGAGATACATAGCTCCGATATTCCTGTGAAAGCCAG

- 20 TGGGAGTTAGGGAGGCTCTTTACAATGACAACATTGCACCAAAGATGGACTCT CTCTCTAAAATGCATTTCATACCAATATTTACTTT

Drosophila EST several including LP04802 (AI260815)

25 Annotated Drosophila genome genomic segment

AE003519

Annotated Drosophila genome Complete gene candidate CG3979 - novel gene with

homology to sodiumdependent dicarboxylate

depondent drea

transporters

30

35

Human homologue of Complete gene candidate

3e-87 4506979

ref|NP 003975.1|pSLC13A2|

UNKNOWN

>gi|2499523|sp|Q13183|NDC1

_HUMAN RENAL

SODIUM/DICARBOXY

40 Putative function

sodium/dicarboxylate transporter

Confirmation by RNAi

Only WT profiles observed

108

Line ID

490/9

Category

Meiotic defects in testis: segregation defects, multipolar spindles

(Mul-02/29)

Reversion

NR

5 Map Position

95C1-8

Rescue ID

I4E

Rescue Sequence

Genomic hit, Accession No. CSC:AC015160

Other results same as 238/20

109

Line ID 660/3

Category Meiotic defects in testis: cytokinesis defects, abnormal spindles.

(Ab-01/03)

Reversion R?
5 Map Position 75E

Rescue ID H8E

Rescue Sequence

20 Genomic hit, Accession No. CSC:AC015160

Other results same as 238/20

110

Example 25 (Category 3)

Line ID 273/18

Category Mitotic defects in brain: metaphase arrest

(overcondensation, very high mitotic index, few polyploids,

metaphase with bipolar spindle)

Reversion NR **Map Position** 75E

10 Rescue ID D1E

Rescue Sequence

5

15

20 GAATCCATATAAAATC

Genomic hit, Accession No. AC015160

Associated ORF

Genscan: >ORF2 predicted sequences

25 >16:57:34|GENSCAN_predicted_peptide_5|1548_aa MLRAVALCVSVVLIALYTPTSGESSQSYPITTLINAKWTQTPLYLEIAEYLADEQA

GLFWDYVSGVTKLDTVLNEYDTESQQYNAALELVKSHVSSPQLPLLRLVVSMHS LTPRIQTHFQLAEELRSSGSCQSFTFAQVGSELACSFNELQKKLEVPLAKDSLDAS VVTYSFDHIFPGSENNTRTVVLYGDLGSSQFRTYHKLLEKEANAGRIRYILRHQLA

- 30 KKDKRPVRLSGYGVELHLKSTEYKSQDDAPKPEAGSTSDEDLANESDVQGFDFK VLKQKHPTLKRALDQLRQRLLQGNDEIAQLKAWEFQDLGLQAAAAIAEIQGDET LQILQYTAHNFPMLARTLLAHKVTDGLRAEVKHNTEAFGRSLNVAPPDGALFING LFFDADTMDLYSLIETLRSEMRVLESLHSNNVRGSLASSLLALDLTASSKKEFAIDI RDTAVQWVNDIENDVQYRRWPSSVMDLLRPTFPGMLRNIRKNVFNLVLVVDAL
- 35 QPTARSVIKLSESFVIHQAPIRLGLVFDARDANEDNLADYVAITCAYNYVSQKKD ARAALSFLTDIYAAVGETKVVTKKDIVKQLTKEFTSLSFAKAEEFLEEDSTYDYGR ELAAEFIQRLGFGDKGQPQALLNGVPMPSNVVTADSDFEEAIFTEIMTHTSNLQKA VYKGELTDNDVAIDYLMNQPHVMPRLNQRILSQEDVKYLDINGVAYKNLGNVG VLNRLSNRDMTATLMDNLKYFGGKKSTELIGRASLQFLTIWVFADLETDQGRDLL
- 40 THALDYVQSGESVRVAFIPNTESSSASSRRNLNRLVWAAMQSLPPTQATEQVLK WLKKPKEKIEIPTQLEDILGSTELHLKMLRVYSQRVLGLNKSQRLVIGNGRLYGPL SSDESFDSADFALLARFSSLQYSDKVRQVLKESAQDVNEEFNSDTLLKLYASLLPR QTKTRFKLPTDLKTDHSVVKLPPKQENLPHFDVAAVLDPASRAAQKLTPILILLRQ VLNCQLNLYLIPVPQHSDMPVKNFYRYVVEPEVQFEANGGRSDGPLAKFSGLPAN
- 45 PLLTQQLQVPENWLVEAVRAVYDLDNIKLTDIGGPVHSEFDLEYLLLEGHCFDAA SGAPPRGLQLVLGTQSQPTLVDTIVMANLGYFQLKANPGAWSLRLREGKSADIYA

111

ISHIEGTNTHHSAGSSEVQVLITSLRSHVVKLRVSKKPGMQQAELLSDDNEQAAQS GMWNSIASSFGGGSANQAATDEDTETINIFSVASGHLYERLLRIMMVSLLKHTKSP VKFWFLKNYLSPQFTDFLPHMASEYNFQYELVQYKWPRWLHQQTEKQRTIWGY KILFLDVLFPLNVRKIIFVDADAIVRTDIKELYDMDLGGAPYAYTPFCDSRKEMEG FRFWKQGYWRSHLMGRRYHISALYVVDLKRFRKIAAGDRLRGQYQALSQDPNS LSNLDQDLPNNMIHQVAIKSLPDDWLWCQTWCSDSNFKTAKVIDLCNNPQTKEA KLTAAQRIVPEWKDYDAELKTLMSRIEDHENSHSRDSAVDDSVDDSVEVTTVTPS HEPKHGEL

5

10 tagcactatatacgccaacttctggggaatccagtcagagctatcccatcaccacgctaatcaacgcgaaatggacgcagacgcc cct at a tot ggaaat cgccg ag ta tot ggccg at gag cag gg ggcct ctt ct gg gat ta cgt tt cgg gg t gacca ag tt ggacang tot ggaaat cgccg gg gat ta cgt tt cgg gg gt gacca ag tt ggacang tot gcggttet caacgaa tatgataccgagtcg caacagtacaatgccgccttggagetggt caagagccatgtgagt tet cccca attgent of the control of the contr15 gtggctcttgtcagagctttacttttgcccaggtgggttccgaactggcctgcagctttaacgagctgcagaagagctggaagtgc egetege caaggatagetteg at gettegetae acetae agettt gate acetatt te cet tigge ag tiggagaa caatac cege act gate acetae agett tiggate acetae agett tiggate acetae aget tiggate acetae acetae aget tiggate acetae acetaeggtactatacggcgatttgggaagctctcaattccgcacctatcacaaactattggaaaaggaagccaatgctggccggattcgtta 20 getttgatttcaaggtgctgaagcagaagcatcctacacttaagagagcgctggatcaactgcgtcagaggcttcttcagggaaac gatgagatcgcccaattgaaagcatgggagttccaggatttgggtctccaggcggccgctgctattgcagaaatacagggtgatg ggcggaggtaaagcataatacggaagcatttggaagaagcttgaatgtagcgcctccagatggtgcccttttcatcaatggactctt cttcgatgctgacacaatggatctgtattccctgattgagacgctgcgctcggagatgcgtgttctcgagagtctgcacagtaataat25 gtgaggggaagcettgecagetcettgetegeettggatetgacgcetecageaaaaaagaattegecategacatecgtgaca ctg cagta cagtgggt caacgat attgaaaacgat gtg cagta ccg cagtggccct catcggtgat ggat ctttt gcgt ccaacctcagatta og tag ceat caegat gege ceata a ctat g tag at caga a a a aggat g cega g ct g ctt ta a g ttt cet cae c g a cat ctat g tag at caegat g caegat g caegat g ctat g a g a caegat g caegat g30 acgcagcagttggtgagaccaaagtggtcacgaaaaaagacatagtcaagcaactaacgaaggaatttacatcattaagctttgc caaageggaggagtteetggaggaagatteeaegtaegactatggeaggagetegeageagtteatteageggetgggatt eggaga caagggacaacct cagg cett gtt gaatggt gtt caatgc cag caacgt t gtgaccg cegatagc gactt cgaggacacgt caggacacgt caggacacacgt caggacacgt caggttgattatetgatgaateaacetcacgtgatgcccagattgaatcagcgaatectaagccaggatgatgtgaaatatettgatattaac 35 at a ctttggtggcaagaag totacggag ctt attggccgag catccctacagt tcctaacgatttgggtgtttgctgatttggaaactgag totacggag catccctacagt tcctaacgatttgggtgtttgctgatttggaaactgag tcctaacgattcctaacgatttggaaactgag tctaacgag tcctaacgag tcctaacgaccagg g to gagatet get cacce at gecet g gae tat g to caa agt g g ag ag t g to gagate g catte at tecaa accet g a comparison of the compaaagetetteegeeteaageeggaggaatettaategattggtttgggetgeeatgeagagtetteeaeeaacteaageeaeggage aggtteteaagtggetaaagaaaceaaaggagaaaattgagataceeacteagetegaggatateetgggatetaeagagetgea 40 ccctttcgtcgatgaaagctttgatagcgccgatttcgctttgctagccaggttcagttctctacagtatagcgataaggtgcgtcagttcagttctacagtatagcgataaggtgcgtcagttcagttctacagtatagcgataaggtgcgtcagttcagttctacagtatagcgataaggtgcgtcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcagttcaggtcctgaaggaatctgctcaagatgtcaatgaggaattcaacagcgatacattgcttaagttgtatgccagcctgcttcccaggca aaccaaaactcgctttaagctaccaacggacttaaaaaccgatcactcggttgtaaaactaccgcccaaacaggagaatcttcccc 45 gccaattgaacttatacctgattcccgtccccagcacagcgatatgcccgtgaagaacttctacagatacgttgtggaaccggag gtcca attcg aggcga atgg aggccg atctg atggtcctttggcca a attcagtg gattgccagcca atcctctgct gacccagcactgtgcacagcgaattcgatctggagtatctgctgttggagggtcactgctttgatgctgctagcggcgctccgcccagaggacttc 5

10

15

25

40

agttggtgttgggtacccagagtcaacctaccttggtagatactattgtgatggcgaatttgggttatttccaacttaaagccaatcca ggagettggtccctacgettgegtgaaggcaaatcggeggatatttatgcaatcagccacattgaaggaacaaatacccatcattcggetggetettetgaagtteaggttettataaceteettgegateecatgttgteaaattaagggtgtetaagaageeaggeatgeag cagtgccaaccaagcagcactgatgaggatacggaaaccatcaacattttetetgtggcatcgggacacttgtacgaacgtettet aaggatcatgatggtttcgctgctaaagcacacaaaatcacctgtgaagttctggttcttgaagaactatctttcgccgcaatttacgg cgatgccatcgtaagaacggatataaaggagttgtatgacatggacctcggaggagcaccctatgcctacacgccattctgcgattcccgcaaagagatggagggettccgattctggaagcagggatactggcgaagccatctgatgggcaggcgttaccacatttccg cettgtacgtggtggacttgaagagattccgcaagattgcggcaggagataggctaagaggccaataccaggcacttagccaggatccgaacagettatccaatttggatcaggacttgcccaacaacatgatccaccaggtcgccatcaaatccctgcccgacgactgg gatcatgagaattcgcatagcagggactcggcagttgatgattcggttgacgattcggtggaggtcaccactgtgacgccttctcat gageceaageaeggegagetgtga

Drosophila Gene Hit rescue sequence and BLASTX with EST and TBLASTN with ORF2: UDP-glucose:glycoprotein glucosyltransferase (U20554)

20 Human Homologue Drosophila EST BLASTX with UDP-GGT: hypothetical protein (AL133051)

several including GH16576 (AI293351)

Annotated Drosophila genome genomic segment AE003519

Annotated Drosophila genome Complete gene candidate ugtUDP-glucose-glycoprotein glucosyltransferase

Human homologue of Complete gene candidate

| CG6850-| IGI_M1_ctg14521_41 |
| D65BCE6EEC187AE3 |
| TRANS:SEPT20T.ctg14521.2 |
| 2 FPC_ctg:ctg14521 |
| FPC_start:1284609 |
| FPC_end:1284696 |
| FPC_strand:+ (1.20E-215) |

Putative function ugtUDP-glucose-glycoprotein glucosyltransferase

Confirmation by RNAi Only wild type profiles observed

113

Example 26 (Category 3)

Line ID 430/5

Category Mitotic defects in brain: metaphase arrest

(overcondensation, polyploidy, metaphase with bipolar spindle)

Reversion NR **Map Position** 98B5-8

Rescue ID 2C2E

10 Rescue Sequence

5

- 15 TAAATGATGTGCCTAAGACTAAGAGTTTAATGAGCATTACTGTCGCGCACTCT ATGTATTATGAATAAAATTCATACAACTTTTGTGGTTTATTATAATATAAAAGT GTGTCAGCTCTACTCGGGGGAAAGTAAGTTTACTTCTTGGCCGCTGGCTTCTTG GCGGCGACCTTCTTCTTGCGGGCGGCCAGCAACTTGGCGCGATTGGCGCAGCC TTGGTGGCCACATTGGCGAAGTGCGACTTGGCCACGTTCTTCTT
- 20 GGCTTGGCCAGCACCTTGGCCACGGTGCGCTTCTCGGCGGCGAGGGCGCAC GACGCTTGAGTACCTCGGCATAAGGGTTCAACTTGATCAACTTGCGCACGGTT GGTAAGGGGGTT

Drosophila EST several including LD45359 (AI513164)

25

Annotated *Drosophila* genome genomic segment AE003763

Annotated *Drosophila* genome Complete gene candidate CG5502 RpL1 - Ribosomal

protein L1

30 Human homologue of Complete gene candidate 1e-126 432359

dbj|BAA04887| (D23660) ribosomal protein [Homo

sapiens]

35

40

Putative function structural protein of ribosome involved in protein

biosynthesis

Confirmation by RNAi Marked decrease in G1 and G2/M indicating fewer cycling

cells

114

Example 27 (Category 3)

Line ID

472/12

Category

5

Mitotic defects in brain: metaphase arrest. Meiotic defects in testis:

segregation defects. Abnormal spindles

(mitotic: High mitotic index, meiotic: Ab-08/24)

Reversion

R?

96C7-9 **Map Position**

Rescue ID

2B6E

10 Rescue Sequence 1

GTCTGACGTTCTCTGAGGGCAAAAGTTTCGAGTTAGTTGAAGGTGAGGGTGCT CGATCACCGATTTGCGGTGAGACGAAAGAAAAGTATGCATTGTTGCGTTGTAA AGAGAGCCGCCCCCTCTTGTTCACATTGTCGCTGAGAACGTATGTTGTGCT TCATCATTTCCTTGTTGATTTCCCTTTGACGTGGCAACTTGACCATGTATGACA 15 ACTCTTTGGTGGTGCCATCTGGAAGGCAGAAATTTGATGTCAACGGTGCTCCC AGCCAGTCCACTCCCCAACTCACCTGCAGCTCCACTTCGATATTAACTCGCA ACATATTAGTGGCGTAGTTGTCACCTGCCGCGGATCCCATTTCCGCTTTGAAAT TTCGCACTTTCGAATATCCGTCCACATTCGATTTGAGAACATCTTCGAAACGTT CAGCGGTGACCCAATCGGGTATTTTGCCAGCCGCCATTGTAGATAATCGGGAT 20 AAGTATTTTGAAATCGAGCAGAAAACACATATACGTCCAGTGTGACGGTCTTG CGTAGACTGATGAAAGCCGAGTATTAGACTCTACACATCTGTGGAGCTTTTTA ATTTCGTAGTGCGCGGCCGATTTCTCTCGATCTTCTCAAAAGCTCCGCTAAT

Annotated Drosophila genome genomic segment AE003751 25 Annotated Drosophila genome Complete gene candidate CG10618 - novel Human homologue of Complete gene candidate none

Putative function

no homologies which indicate function

30

Confirmation by RNAi

Only wild type profiles observed

115

Example 28 (Category 3)

Line ID 571/15

Category Mitotic defects in brain: metaphase arrest

(overcondensation, few anaphases, some polyploids)

Reversion NR **Map Position** 93D

Rescue ID 2A8E

10 Rescue Sequence

5

30

GGCGCCCTCACATTTGTTGTTGTCGCTGCTCACAGCTCCACCACCATTTGC ACAGTTATATTACCTCGCTCAAGTCGCCCCTCTCCCTCTCGCCCACTCGCTGTG TCAATCGAATTAAAACGAATGCTCTTCGGCGAATAATTGGGTTTAGATACTTT TCCAGCAGACAAAGTTGTATTTTTTGCACTTCTTATTGATATTAGGCAAAACGC

- 15 ATCGGCCGAATCACACGCACACAAAGCACACACGCGAGCAGCGGTTTTTCAA
 TCTGCAGTACACCAAACAACACACACTATTTCCTAATGCCTGTTCTTATCCCTC
 TGATATTCCCAATGAATCGCTGGGCAATTGGCGATTCGAACCGATTTTCACTT
 GGCTCTTTGTTTTATTTAATTTTCACCGAAACGCTCTCACACGCAGAGACGCTT
 TTGCTCGTTCGCTGATGCTTCTGCTGCAATACACACCACCTACGAAACGACC
- 20 AAGGAAATTGTATCTATGGGCTGTGTATCTGTTTCTACGCGGCACGCGCTGC ACGTCCGCTCGCTTCGGGTTTTCGAGAGAGAATATAACTTTTTCGATACGGTA CGGTAAACGAATTCCGCGGAATTAATTCTTGAAGACGAAAGGGCCTCGTGATA CGCCTATTTTATAGGGTAATGCATGATAATAATGGGTTCTTAGACGTCA
- 25 *Drosophila* **EST** LP07504 (AI294185), LP06548 (AI293427)

Annotated Drosophila genome genomic segment AE003734

Annotated Drosophila genome Complete gene candidate CG15802 - novel homology

to Doom, a product of the Drosophila mod(mdg4) gene, induces apoptosis and binds to baculovirus inhibitor-ofapoptosis proteins

35 Human homologue of Complete gene candidate none

Putative function inducer of apoptosis

Confirmation by RNAi Only wild type profiles observed

116

Example 29 (Category 3)

Line ID 736/15

Category Mitotic defects in brain: prometaphase arrest

(overcondensation, fewer anaphases, metaphase with bipolar

5 spindle)

Reversion NR **Map Position** 73C

Rescue ID H5E

10 Rescue Sequence

15

30

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20 Genomic hit, Accession No. CSC:AC014181

Annotated *Drosophila* genome genomic segment AE003526

Annotated Drosophila genome Complete gene candidate CG3971 baldspot - with

homology to membrane

25 glycoprotein

Human homologue of Complete gene candidate CG3791-9e-08

4680391emb|CAB41293.1| (AL034374) dJ483K16.1 (novel protein) [Homo

sapiens]

Putative function membrane protein, function unknown

Confirmation by RNAi Slight reduction of G1 and G2/M peaks indicating fewer

cycling cells

117

Example 30 (Category 3)

Line ID 82/24

Category Mitotic defects in brain: metaphase arrest

(condensation, no polyploidy, no anaphases, metaphase with

bipolar spindle)

Reversion NR Map Position 100D

10 Rescue ID 2E3E

Rescue Sequence

5

45

GGTCAAGCCCGATGGCGTCCAGCGCGGGCTCGTCGGCAAGATCATCGAGCGC
TTCGAGCAGAAGGGCTTCAAGCTGGTCGCCCTGAAGTTCACCTGGGTAAGCGG
ATAATTGAATTAGGAAGAAATCAATAGATATACATACGTGGAAACGGGTTGC

15 CCCACGCGGGGTTGCTATCGGACCTAACCTCAAAGGCTGGGTGCAGGCGTCAT
CGCGGAATGACATGTGTTTAGAGGTCAGAACTGCAATTAACTGATAACGAACC
GTTTTGTAACCAGGCCTCCAAGGAGCTGCTGGAGAAGCACTACGCTGATCTGT
CCGCCCGCCCCTTCTTCCCCGGACTCGTGAACTACATGAACTCCGGCCCCGTG
GTGCCCATGGTGTGGGAGGGTCTGAATGTGGTCAAGACCGGTCGCCAGATGCT
CGGCGCCACCAACCCCGCCGACTCGCTGCCCGGCACCATCCGCGGTGACTTCT
GCATTCAGGTCGGACGCAACATCATCCACGGCTCCGATGCCCTGCCC
GAGAAGGAGATCGCCTGTTCAACGAAAAAGGAGCTGGTCACCTTGCC
GAGAAGGAGATCGCCTGTGGTTCAACGAAAAAGGAGCTGGTCACCTGGACCCC
GG

25 Genomic hit, Accession No. CSC:AC012727

Associated ORF

Genscan ORF1 predicted sequences >16:43:49|GENSCAN_predicted_peptide_7|172_aa MKLLMLGTILAFFSVISATMAANKERTFIMVKPDGVQRGLVGKIIERFEQKGFKLV

30 ALKFTWASKELLEKHYADLSARPFFPGLVNYMNSGPVVPMVWEGLNVVKTGRQ MLGATNPADSLPGTIRGDFCIQVGRNIIHGSDAVESAEKEIALWFNEKELVTWTPA AKDWIYE

>16:43:49|GENSCAN predicted CDS 7|519 bp

atgaageteetgatgeteggeacaattttggcattetttetgtaateteggegacaatggeggetaacaaggagaggacttteateat ggteaageeegatggegteeageggggetegteggeaagateategagegettegageagaagggetteaagetggtegeee tgaagtteacetgggeeteeaaggagetgetggagaageactaegetgatetgteegeeegeeettetteeeeggactegtgaa etacatgaacteeggeeeetggtggeeeatggtgggagggtetgaatgtggteaagaeeggeeeae eaaeeeegeegaetegetgeeeggeaceateegggtgaettetgeatteaggteggaegeaaeateateeaggeteegatge egtegatgeeeggtgaettetgeatteaggteggaegeaaeateateeaeggeteegatge egtegagtetgeeggaagaaggaggategeeetggtteaaeggaaaaggagetggteaeetggaeeeeggeeeaagaetgg atetacgaatag

Drosophila Gene Hit rescue sequence and TBLA: abnormal wing disc (awd) (X13107) Human Homologue BLASTX with awd and TBLASTN with ORF1: tumor metastasis

inhibitor nm23-H2 (A49798) non-metastatic cells 2, protein (NM23B) (P22392) and nucleoside diphosphate kinase B.

118

Drosophila EST

several including LP05977 (AI257573 similar by TBLASTX to X92956 B.taurus mRNA for nucleoside diphosphate kinase (NBR-A)

5 Annotated Drosophila genome genomic segment AE003779

Annotated Drosophila genome Complete gene candidate CG2210 - awd abnormal

wing discs nucleoside diphosphate kinase

10 Human homologue of Complete gene candidate gi4505409

1A5C3F84D7AD272C |ref|NP 002503.1| nonmetastatic cells 2, protein (NM23B) expressed in [Homo

sapiens] (1.90E-61)

15

20

human nucleoside diphosphate kinase, transcriptional regulation of **Putative function** c-myc expression.a candidate suppressor of tumor metastasis

Confirmation by RNAi

Only wild type profiles observed

119

CATEGORY 4: ANAPHASE DEFECT

Example 31 (Category 4)

Line ID 1132/8

5 Category Mitotic defects in brain: anaphase defects

(overcondensation, high polyploidy, some lagging chromosomes)

Reversion ?

Map Position 86F3-6

10 Rescue ID 2C3E

Rescue Sequence

35

40

GGCCGGÂGGTACCATTTTGGTAGGACCGTTTTTCGGGCCAACGAAAATACCAC AAGACGGCAGCGATAATAGTGTTTTTTTGCTTCAAATGTAGTATGGCTACGCAA CTCACATATGGTTAAGAACTTCGCTGTTTTATTTGGTGGTTAAACTAGCTAAATA

- 15 CAATAAGAGTGGCAACGCCGTCACGTTTTCTACATGTATTTTACTTGGCGTAGT GCGCCAAGCTTATAAACCACAGTTGGGCGGTTCTTTTGAATTGTTTAATTTACA CCCCACTATGAAACTTATTAGCCTTCTTTATTTATTTTATATTTTATTTTTAGGA AGAATACGTTTACTCAAGGTTCGCAGCTTGTCAATCAGTATTCGCAAATATCA ATAATAAAAGGCATCAATTTTCCAATCAGCAGTTGAAAAGAACTCCCCTCGAC
- 25 Genomic hit, Accession No. AC007805

Drosophila EST several ESTs including LP09688 (AI295922)

Annotated *Drosophila* genome genomic segment AE003693

30 Annotated Drosophila genome Complete gene candidate CG6929 - Lk6 kinase

Human homologue of Complete gene candidate gi4505191

DB39E49EC0BED990 |ref|NP_003675.1| MAP kinase interacting kinase 1 [Homo sapiens] (6.20E-113)

and gi9994197

551A82FA3D09FD58 |ref|NP_060042.1| G protein-

coupled receptor kinase 7 [Homo sapiens] (1.70E-106)

Putative function Protein kinase associated with microtubules

120

Confirmation by RNAi cells

Complete loss of G1 and G2/M indicating fewer cycling

.

121

Line ID 483/19

Category Meiotic defects in testis: segregation defects

Reversion ? Map Position 86F

Rescue ID H2S

Rescue Sequence 1

5

20 Genomic hit, Accession No.

CSC:AC018284

Drosophila EST

several including GH28825 (AI517767), LP04213

Other results same as 1132/8

WO 01/72774

122

Example 32 (Category 4)

Line ID

1422/14

5 Category

Male and female sterile, small wings, meiotic defects in testis:

PCT/GB01/01297

segregation defects, elongation defect

Reversion

NR

Map Position

90B4-8

10 Rescue ID

2F1E

Rescue Sequence

GGCCAGCTGCTCAAACATTCTGCAGCTATTTGGCCGCCAGCGAGTAGAACGAT ATTGCCAAATATTTTATAATAGTAACCAATACGTTACCAGTATGACCGCGCCG ATAACGATAGAAAATACCACACGGTCTAAAAGTAAATACCATTTGGGGTATTC

- 20 ATAAAACTGGTAATTAACAAAAGTAAAAAGTTACTTAACTTATACAAAAATAT TTAGTTATTGNATTCAATAATAAGATGGTAATAATAAGATGGTAAGATAGTAAT ATTTTAATAATTGAATTCATCACACATGCTGGTGCACGTTCCACAACTTACAA TCAAACGAAA
- Annotated *Drosophila* genome genomic segment AE003718

 Annotated *Drosophila* genome Complete gene candidate CG7623 novel with homology to UDP-galactose transporter.
- 30 **Human homologue of Complete gene candidate** 2136348 UDP-galactose transporter related isozyme 3 human >gi|1669564|dbi|BAA13527| (1e-36)

35

Putative function sugar modification protein

Confirmation by RNAi Slightly reduced G2/M

123

Example 33 (Category 4)

Line ID 1479/10

Category Mitotic defects in brain: anaphase defects

(overcondensation, anaphase bridge, metaphase with swollen

chromosomes and bipolar spindle)

Reversion NR **Map Position** 69F3-7

Rescue ID 2D6E

10 Rescue Seguence 1

5

CCACGGGCAAATGTGGTCCGGAGGTCCACGACAACGTGCCGCTGACCATATC
CCAGATTGAGCGCGCAACTCAGGATCCGGAGAACGAGAATGTGTTCATCACA
GACGACGTGCATCCGATTCACTTCTGCACCTGCATCATCTACGCCTTTGTAACT
GGCAATGGAACGCACAACGAGTCGTTCATGAAGTTCATGATCGATGATGGCA

15 CCGGCTCCCTGGAGGCCAGCATCACCAAAAAACCCTTCAATGGACGCGTGATC
AGCAGCCTGTACAGTGAAGCCAGTTCGCTGGCCTCCTCGAGGCCTACAAGA
GCATTGCCGTGAGCATGATGCGGCTGCTGCAGGTCTCCATGGAGTACATTGAT
CCCACGCGCATCTCGAGGGGCCACAGCCTATTCCTGCGCGGGTCGTCCGAATAG
GTTCCGCGGCAAGATGGGTCTGGACGCTTTTCAGTTCTTCATAGACAGCGGCC
20 GATCGCGGAATATGGAAATTGGCTTCGTGGACTACCTAACCGACTGGCAACG
AAGGCATAAAACAATGCAAAATAC

Rescue ID 2D6P

Rescue Sequence 2

25 GCCCGTGGACTTTTCACTCTGTTGATTCTTGCGTATCACGAAGTTATCCAGCTG
GCTTTCTATGTCCTCGAAACTCTGATTAAAATCCATTCTATTTGCTTAGTCTGC
GATTTCAAAGGGGATTTCTTTATTGCAGTGCATTTTGCATTAGCGCCAAAAA
AAAAAGTTGTGAGCATGGGCGTAGACTTCGTATTTTCTTACAAATAATATTA
ATTAAAATTAATTTTGTGAGCAATTTTCACACAATTGTATTATAAGTTAAAACC

30 AGGGTCACATTAATTTGCAGAACCGCGCAATATTTTCTTTTTAACCCCCTTACA
AATTTTCAGTTGTTTTGACTACGCCCCTGCTAATTTTTACTTATTAAATTCAAA
GTCTAAAAACATTGTCACCAGATAATACGAGTATACACTATATGGACAAACGT
AAAATCGTTAATAGAATATATATATTCAACCATTATTTCACCACCGAGAGAAA
TTCATTTGCACAAAACGCCAGGTTGGCAGCACCATCATTGCGCACAGCAAGTG

35 GGCAAACTCGTTGTATCGCTTG

Genomic hit, Accession No. AC007328

Associated ORF

40 Genscan ORF1 predicted sequences >17:42:01|GENSCAN_predicted_peptide_2|1507_aa MKLAPTVKLNNGYEMPILGLGTYNLKKSRCEAAVCHALEMGYRHIDTAYLYRNE GIIGKVLAKLIGDQKLKREQVFLVTKLWDIYHEPKMVKYACDMQLKLLGVDYID LYLMHSPVGVDYISDEDLMPHENGQLRTNDVDYVDTYRSMEQLVHLGLVRSLG LSNFNANQLKRLLENCQIKPANLQIECHPELVQVPLIELCKFHNITVVAYSPLGRSQ

45 TCNPLPDYYTDSKLLALAAKYGKTPAQIILRYLSKDNEGEAAVKHAIDVGYRHID TAYFYQNEAEVGKAIRDKIAEGVVKREDIFLVTKLWNIFHDPERVEGICRKQLSNF GLDYIDLYLMHMPVGYKYVDDNTLLPKNEDDVLQLSDVDYLDTYKAMEKLVKL

124

GLRIEQLAGLSHLSTHSDGMQFRIRMFLTFQRGGPSHNNMQQQQQRGGGSGTDF YNQQRDRRDSGRQMDNNYSNNYNNNNNNQRNRGGGNGMQQQQRGGNGGSGG GGGNGGGNNPAWNMHRGNQNSNNMMNMRNRGMGSRGPMRPNQVHLLVTHT AIDGLLNPGFHILQGYRPQSANNQNKPRNKIKFEGDFDFEQANNKFEELRSQLAKL 5 KVAEDGAPKPATNATAATATATNEQVGEKVEGVHTLNGETDKKDDSGNETGAG EHEPEEDDVAVCYDKTKSFFDNISCEAAQDRSKNKKNDWRQERKLNTETFGVSS TRRGSVAHQLNVFQAVTADATNTTTIMATAALTRDMEERQATTGTIIAWVGGGG NFRNRSNNRNNGGGRGGNGMPNITNGNTAAALKAANNAAGHGSNATDSSAPNA TTATTKSTSLLPEQTQQVAAVSLPVLLPSIGWLFIVMDGPPDIPRSADIAILFVSFEQ 10 SVLFLKFHKRYNEFAHLLCAMMSFEDIESQLDNFVIRKNQQSEKSTGKCGPEVHD NVPLTISQIERATODPENENVFITDDVHPIHFCTCIIYAFVTGNGTHNESFMKFMID DGTGSLEASITKKPFNGRVISSLYSEASSLASSEAYKSIAVSMMRLLQVSMEYIDPT RISRGHSLFLRGRPNRFRGKMGVCTNATAPSVSSINRILRNRAAERAAAEFARAAS YGYAIHPTHPHPYTSFPTWPAHHPLWGAVPLATPPGGGPAGAGGALOPGGSGSSY 15 GSDGNMSSNPNSSNSNTTHSNGHNTNSGSGCGDSSAGSGRLSLPALSPDSGSRDS RSPDADANRMIDIEGEDSESQDSDQPKFRRNRTTFSPEQLDELEKEFDKSHYPCVN TREKLAARTALSEARVQVWFSNRRAKWRRHQRVNLIKQRDSPSTSSSPTPLVNPV VSPVSPIPVPVPVAVPESGQQKQPYPYSTSNMCNTSSSSSNSQPCNTINPGSKMSSK TSSVSSNQHMEEPAAAVATASPTASAPLSMGGENSAFRALPMTLPMPMTLPTASA 20 AAFALSFARQYIAKTLLGSPPRSQPPTTNQHKPEPNREFLNEACSSAASVQNSTTP ATTADTPTAKSAMCVHCEKKGGAMEWM

>17:42:01|GENSCAN predicted CDS 2|4524_bp

atgaagctcgctccgactgttaagctaaacaatggctacgagatgccaattctgggcctaggaacctacaatttaaagaagtctcgc 25 tgtgaggetgeegtgtgeeacgeectegaaatgggetateggeatatagaeacegeatatetgtaeaggaatgaaggeattatag gaacccaagatggtgaaatacgcctgtgatatgcaattaaagctactgggcgtggactatatagatctatatctgatgcattcgccg gtgggggtggactacatctctgatgaagatctgatgcccacgagaatggccagctgaggaccaacgatgtggactatgtggac 30 attactggaaaactgccaaatcaagccggcaaacctacaaatagaatgtcatccggaattggtgcaagtcccattaattgagctctgaaactactggcgttggcagcgaaatacggcaagacaccagctcaaatcatcctaagatacttgtcgaaggacaacgaaggcgaa gccgctgtgaaacatgcgattgatgtgggctatcgtcatatagatacggcctatttctaccaaaacgaggccgaagtgggcaagg cgattcgggacaagatcgcagaaggtgtggtcaagcgagaggatatatttttggtcactaagctttggaacattttccacgatccag 35 caaatatgtagatgacaacaccctgctgcccaaaaatgaggacgatgtgctccaactgagcgatgtcgactatctggatacgtaca aagccatggaaaagctggtaaaactgggcctgcgtatcgaacaacttgctggcctgagtcatctttcaactcattcagatggcatgc agtttcggatacggatgtttctaacattccaacgtggcggacccagccacaacaatatgcagcagcagcagcagcagcggcg 40 ggcggcggcggtggaaacggaggtggaaacaacccggcctggaacatgcatcgcggcaaccagaactcgaacaacatgatg ggtttattaaaccctggctttcacattttgcagggctatcgtccgcagtcggccaataatcagaacaagccgcggaacaagatcaa gttcgagggcgacttcgatttcgagcaggcaaacaacaagttcgaggaactgcgctcccaactggccaagctcaaggtggccga 45 ggcgttcacacactgaatggcgagaccgacaagaaggatgattctggcaacgagaccggcgctggagagcacgagcctgaggaggatgatgttgctgtgtgctacgacaagaccaaatcgttcttcgacaacatctcgtgcgaggctgcccaggatcgcagcaagaa caa gaa gaa c gat t g g c caa g a g t g a a cac g g a g a c c t c g a g t g t c c t c a c a c g a c g t g g c a g t g t c c c a c a c g a c g t g g c a g t g t g c t c a c a c g a c g t g c a g t g c a c a c g a c g t g c a c a c g a c g t g c a c a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a c g a

atcaactgaatgtattccaagcagttaccgcggacgcaaccaatactacaacaataatggcaacggcggcattaactcgggatatg aacaacggcggcggtcgtggcggaaacggaatgccaaacatcaccaatggcaacacggctgctgcgctgaaggcggccaac aatgetgetggecaeggatceaatgecaeggactceagtgeaccaaatgecacaaeegegaegacaaagtegaegteeetettg cagacattccaagatcggcagatattgcgattctcttcgttagttttgaacaaagtgtacttttccttaaatttcacaagcgatacaacg agtttgcccacttgctgtgcgcaatgatgagtttcgaggacatagaaagccagctggataacttcgtgatacgcaagaatcaacag agtgaaaagtccacgggcaaatgtggtccggaggtccacgacaacgtgccgctgaccatatcccagattgagcgcgcaactca ggatccggagaacgagaatgtgttcatcacagacgacgtgcatccgattcacttctgcacctgcatcatctacgcctttgtaactgg caatggaacgcacaacgagtcgttcatgaagttcatgatggtggtggcaccggctccctggaggccagcatcaccaaaaaaacc cttcaatggacgegtgatcagcagctgtacagtgaagccagttcgctggcctcgtccgaggcctacaagagcattgccgtgagc atgatgcggctgctgcaggtctccatggagtacattgatcccacgcgcatctcgaggggccacagcctattcctgcgcggtcgtc egaataggtteegeggcaagatgggtgtetgeaceaatgecactgeteetteggtgagcagcatcaategeatattgegtaatega agtttccccacttggccggcgcatcatccgctgtggggagccgtgcccctggccacgccacctggtggcggccctgctggagccggtggtgcactgcagccggcggcagtggcagcagctatggcagtgatggcaacatgagctcaaatcccaatagcagcaaca gcaacaccaccacagcaatggccacaataccaacagcggcagtggatgcggggatagtagtgccggaagtggacgcctctc cgaggacagcgagtcgcaggacagtgaccagccgaagttccggcgcaatcgcaccaccttcagtccggagcagctggatgag etggagaaggagttegacaagtegeactatecetgegtgaataeeegegagaaaetggeegeeeggaeggeactgagegagg ccagggtgcaggtttggttttccaacagacgagcgaaatggcggcgccaccagcgggtcaacttgatcaagcagcgcgactcg ccetcgacatcgagctcacccacgccgttggtcaatccggtggtcagtccggtcagtccaatcccagttccagttccagttgcagtt ccagaatctggccaacagaagcagcatatccgtacagcaccagcaacatgtgcaacaccagcagcagcagcagcagcaacagtc aaccgtgcaacaccatcaatcccggcagcaaaatgagcagcaaaaccagcagcgtcagcagcaaccagcacatggaagagc cagcagcggtggccactgcctcaccacagcatcagctccattatcaatgggcggtgagaacagtgcatttcgcgctctgcc catgacettgecgatgeccatgacettgeccaeggeateggeggegteteggeteagettegeegeeagtacatagecaa gacgetteteggttetecagateceagateceagecaceaceaceaceageataagecegagecaaategegagtteeteaat gaagcetgcagetccgcagcatctgtccagaattcgacaacgccggcaacaaccgcagatactcctacagccaaatcagcaatg tgcgtgcactgcgagaaaaagggaggggccatggagtggatgtga

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Drosophila Gene Hit BLASTN with rescue sequence 2: Histone acetyltransferase GCN5 (AF029776) very small match at end, TBLASTN with ORF1: middle domain histone acetyltransferase GCN5 (AF029776). Genomic matches histone acetyltransferase

35

Annotated Drosophila genome genomic segment
Annotated Drosophila genome Complete gene candidate CG4107 -Pcaf /GCN5 histone
acetyl transferase
transcriptional activator
protein
Human homologue of Complete gene candidate

Human homologue of Complete gene candidate

gi6382076
72F516F8BD10CD0C
|ref[NP_003875.2| p300/CBPassociated factor [Homo

sapiens] (1.20E-197)

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Putative function Transcriptional activator

126

Confirmation in RNAi Only wild type profiles observed

127

Example 34 (Category 4)

Line ID 184/5

Category Mitotic defects in brain: Anaphase defects.

(overcondensation, aneuploidy, some lagging chromosomes and

breaks)

Reversion R **Map Position** 71B

10 Rescue ID C4E

Rescue Sequence

5

Genomic hit. Accession No. CSC:AC019852

25 Associated ORF

Genscan ORF1 predicted sequences >22:43:26|GENSCAN_predicted_peptide_2|1003_aa MAPKKSTIVLNVEQFIHDIEERPAIWNRNFHCNKAFLEQMWDELSGAHKLPKIVL KAKWKGLRDNFRVEYKRIPRADNGDFMVDPATFESKWLHYYALLFLTDHMRHR LPKNEQDQSFYFSQQSEDCEKTVVEPDLTNGLIRRLQDSDEDYDEEEMEADGEAS

- 30 EATMEETMPTPPAAHQMNQVSTTPLATGALRAQEEAHQHALIKAGLLRAQLMEL EKEAEDLSRKPPPPQQMTSPVAPSLQVLVEPPAAHCSPPPMVTTTSAQVQQPGSA AVLAPATTTSASSVSSNGAPMGGKRSVSPPPLYNKAHHPLATLAAAHLAAKDRN EDFGPTSAVGGNGDHLSFTQHSYANGLIPALKLKRPRLSEDSNFNGSSTMDTPLVP EDDDYHYLLSLHPYMKOLTAAOKLRIRTKIQKLIFKELYKEDLEESNLDREVYVL
- 35 DDGAEVDLDLGNYERFLDVTLHRDNNITTGKIYKLVIEKERTGEYLGKTVQVVPH ITDAIQEWVERVAQTPVQGSSKPQVCIVELGGTIGDIEGMPFVEAFRQFQFRVKRE NFCLAHVSLVPLPKATGEPKTKPTQSSVRELRGCGLSPDLIVCRSEKPIGLEVKEKI SNFCHVGPDQVICIHDLNSIYHVPLLMEQNGVIEYLNERLQLNIDMSKRTKCLQQ WRDLARRTETVRREVCIAVVGKYTKFTDSYASVVKALQHAALAVNRKLELVFIE
- 40 SCLLEEETLHSEPSKYHKEWQKLCDSHGILVPGGFGSRGMEGKIRACQWARENQ KPLLGICLGLQAAVIEFARNKLGLKDANTTEIDPNTANALVIDMPEHHTGQLGGT MRLGKRITVFSDGPSVIRQLYGNPKSVQERHRHRYEVNPKYVHLLEEQGMRFVG TDVDKTRMEIIELSGHPYFVATQYHPEYLSRPLKPSPPFLGLILASVDRLNQYIQRG CRLSPRQLSDASSDEEDSVVGLAGATKSLSSLKIPITPTNGISKSCNGSISTSDSEGA

45 CGGVDPTNGHK

128

>22:43:26|GENSCAN_predicted_CDS_2|3012_bp

atggcgccaaaaaagtccaccattgtgctcaatgtggagcagtttattcacgacatcgaggagcgcccggccatctggaaccgca atttecaetgeaacaaggeetteetegageagatgtgggaegagetgageggagegeacaaaetgecaaagategtgeteaagg 5 atcagtcattttacttcagccagcaaagcgaggactgtgaaaagacagtggtggagccggatttaacaaacggtctaatacgtcgt ctgcaggacagcgacgacgaggattacgacgaggaggaaatggaggcgacggagggtagcgaagccaccatggaggaaac gatgcccacgccaccggctgcgcatcaaatgaatcaagttagcaccaccactggccaccggagctttgcgagcccaagaag aggcacatcagcacgctttaattaaggcaggattactccgcgctcagttgatggagctggaaaaggaggcggaggacttgagca 10 gaaagccacctccgccacagcaaatgacatctccagtggcaccctcactacaagtgctagtggaaccaccagccgcacactgtt ctccaccgccaatggtgaccaccacatccgcacaagtacaacaaccgggctcagcagctgttctggcgccggcaacgaccaca teegegteatetgtateetegaatggagegeeaatgggeggeaagagatetgtgtegeeacegeetetatacaacaaageacace atccgctggccactctggcagcagcacatcttgcggccaaagaccgaaatgaggatttcggacccacctctgctgtaggaggaa acggagatcacctgagcttcactcaacactcctacgccaatggactgatacccgcccttaagctgaagcgcccgcgtctctccga 15 ggatagcaattttaatggttcctcgacaatggacactccgctcgtaccagaggacgatgactaccactacttgctcagcctacatcc gtacatgaagcagctgaccgcagcccagaagctgcgcatacgcaccaagatacaaaagctcatcttcaaggaactctacaaaga agatettgaggagteeaacetagategegaggtttaegttttggacgatggegeegaggtggatetggatetggaaactatgaae ggtttttggatgttaccctgcatcgggacaacaacataaccaccggaaaaatttacaagttggtcattgagaaggagcgcactggc gagtacttgggcaaaacggttcaagttgtcccacacatcactgatgccattcaggaatgggtggagcgctggcccagacaccc 20 gttcagggatettcaaagccacaggtgtgcategtggaattgggaggaacgattggtgacatcgaaggcatgcetttcgtagagg cette c g teagttt cagtte c gegtaa ag ag ag ag aactte t g tt t g cette g tt g cette g tt g cette g g teagtte c g tt g cette g g teagtte g tt g cette g cette g tt g cette g cette g tt g cette g cette g tt g cette g cette g tt g cette g cette g tt g cette g tt g cetaacccaagaccaagcccacacaaagttcggtcagagaactgagaggatgtggcctgagtcccgatttgattgtctgccgatcgga gaaacccattggactggaggtcaaggagaagatcagcaacttttgtcatgtggggccggatcaggtgatatgcatccacgatttga actccatttatcatgttccgctgctgatggagcagaatggtgttattgaatacctaaatgagcgcctacagcttaatatcgacatgagc 25 aagaggaccaaatgcttgcagcaatggcgagatttggcgcgtcgaacggagaccgttcgccgtgaagtttgcatcgccgtcgtg ggaaagtacaccaagttcacggattcgtacgcctccgtagttaaagccctgcaacatgccgccctggcagtgaatcgcaaactgg aactggtetttategagtegtgeetgetggaggaggaaactttgeattetgageegageaagtaeeacaaggagtggeagaaget atgcgatagccatggcatcctagtccccggtggattcggttcccgtggaatggagggcaagattcgtgcatgccaatgggcgcga 30 gcaaacaccacagaaatcgatccgaacacagctaatgccttggtcatcgatatgccagagcatcacacgggtcaattgggcggc actatgcgcttgggcaagcgaataactgttttetetgatggteetagtgteattegecagttgtatggcaateegaaaagcgtgcagg cgacaaaactaggatggaaatcattgagctcagcggtcatccctactttgttgccacccaatatcatccagagtacttgtcgcggcc tetga ag cegtege cte ettte ctegge ctgate ctgge cteagtggategattgaace aat at atteag eg eg gtt geege ctgtegategat ctgategategategat can be a similar at a similar35 cccgccagctatccgacgcatcctcggatgaggaggacagtgttgtgggcttggccggagcaacaaaatcgctgagctccttgaaaattcccattacacccacaaatggaatatcaaaaagttgcaatggtagcataagcacttccgacagcgaaggtgcctgcggag gcgttgatcctaccaatggccataagtaa

Human Homologue TBLASTN with ORF1: CTP synthase (CTPS) (NM_001905.1) Drosophila EST LD27370 (AA941993)

Annotated Drosophila genome genomic segment AE003532

Annotated Drosophila genome Complete gene candidate CG6854 - novel protein, possible CTP synthase?

Human homologue of Complete gene candidate

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gi4503133 C33BD849A0044697 |ref|NP_001896.1| CTP

129

synthase; cytidine 5-prime triphosphate synthetase [Homo sapiens] (8.40E-217)

5 **Putative function**

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Enzyme important in the biosynthesis of phospholipids and nucleic acids, and plays a key role in cell growth, development,

and

tumorigenesis. The region of the human gene is the location of

breakpoints involved in several tumor types

Confirmation by RNAi

Loss of G1 and G2/M peaks indicating fewer cycling cells

130

Example 35 (Category 4)

5 **Line ID** 225/27

Category Meiotic defects in testis: segregation defects

Reversion NR **Map Position** 90D

10 Rescue ID 2D2P

Rescue Sequence 1

Rescue ID 2D2E

15 Rescue Sequence 2

- 25 CTACATTTTGAATGTTCACAATGAAAATTGCTGGGGAAGCTAGTGAACAACCA TTTCGCCATAATTTACACTATCTAAGCTTTTATTTTTAGCCACATGATATATGC ATGCA

30 Genomic hit, Accession No. AC008361

Associated ORF

Genscan ORF1 predicted sequences >20:36:39|GENSCAN_predicted_peptide_2|515_aa MSSTIRLQTSSCQCCKLYKYERHPNKPNLQPTPIPNYPCEILHIDIFALEKRLYLSCI

- 35 DKFSKFAKLFHLQSKASVHLRETLVEALHYFTAPKVLVSDNERGLLCPTVLNYLR SLDIDLYYAPTQKSEVNGQVERFHSTFLEIYRCLKDELPTFKPVELVHIAVDRYNT SVHSVTNRKPADVFFDRSSRVNYQGLTDFRRQTLEDIKGLIEYKQIRGNMARNKN RDEPKSYGPGDEVFVANKQIKTKEKARFRCEKVQEDNKKNRNGKAAGGKGKTR RVARGAQIYQNWAICRNLFLFLSLACCRVCKVCDIVVEFRKGTNAVVNVQIREAI
- 40 SHVFHKEDIVIDVQESKEWCIWTDDQVQSPLPELENLWHELWIGPSHAYLIDQIVD LFENLLEKYNVQVVDVVRFNFLHRALVVVIISGIIIIIIMIIGVSGGQRTNAFSHHRS QRSAIGGDPQQKDSAVQQVQARSSDAFCQIPHRSPRFPGRSQLIPKPNREILRNASA TKNLLFRIRSQ
- 45 >20:36:39|GENSCAN_predicted_CDS_2|1548_bp atgtccagtacgatccgtctgcaaacttcctcatgtcagtgttgcaaactctacaagtacgagagacaccctaacaaaccaaaccta

131

caacctacgccaattcctaactacccatgtgaaatacttcacatcgacatttttgcgctcgaaaaaaggttatacctaagttgtattgac aaatttagcaagtttgccaaacttttccatctgcagtcaaaagcatctgtgcatttgcgagaaactttggtggaggccctacattacttc accgccccta agg tettgg ttt cgg at aac gag cgag gg tt gtt at gcccca cag t get caact at ett cgg tet ctag at at cgat ctag at a cgccccta agg tett caact at ett cgg tet ctag at a cgccccta agg tett caact at ett cgg tett caact at ctag at a cgccccta agg tett caact at ett cgg tett caact at ctag at a cgccccta agg tett caact at ctag at a cgcccca agg tett caact at ett cgg tett caact at ctag at a cgcccca agg tett caact at ctag at a cgccccca agg tett caact at a cgcccca agg tett caact at ctag at a cgcccca agg tett caact at a cgccca agg tett caact at a cgccca agg tett caact at a cgcccca agg tett caact at a cgccca agg tett caact at agtattatgctccaacccagaagagcgaagtaaatggtcaagtcgagagattccactctacgttcctagaaatttatcgttgccttaaa 5 gatgagetccctacettcaaaccegttgagetggtacacatagcagtggacegetacaacacttcegttcacteggtaacgaateg aaaaccagcagacgtttttttcgaccgctcgtcaagggtaaactatcagggtctgacagatttccggcggcagactttagaggacat caagggettaattgagtataagcaaattagaggtaatatggeteggaataaaaatagggacgagccaaagtettatgggeeggga gatgaagtttttgttgcaaataagcaaataaaaacaaaggaaaaagcgaggttcagatgcgaaaaggtacaggaagacaacaag aaaaatcgcaacggaaaagcggcggtggaaaggggaaaactcgcagagtagcccgtggagctcagatttatcaaaactggg 10 ccaacgccgtcgtgaacgtgcagatccgtgaagctatcagccatgtgttccataaagaagacatagtcatcgatgtccaggagtcc tag ccat gcgtacct gate agatt gtcgat ctcttcgaaaaat ctgctcgaaaaat at aat gtgcag gtt gtcgat gtag ttcggttcaattteeteeategegetetegtagtegtgateatttegggtateateateateattateateatgateateggegttagegggggeea 15 aagaacaaatgccttttcacaccaccgatctcagcgatcagcgatcggcggcgaccctcaacaaaaagattcagcggtgcaaca ggtgcaggcacgatcttcggatgccttttgccagataccccaccgatctcccaggttcccagggcgcagccaacttattccgaagc caaatcgagaaattcttcgaaacgcgagtgccaccaaaaatttattgtttcgaattcgcagccagtga

Drosophila Gene Hit BLASTN with rescue sequence: couch potato (Z14974).
 Human Homologue BLASTX with couch potato: RBP-MS/type 2 (RNA binding motif family)(D84108)

25 Annotated *Drosophila* genome genomic segment AE003720
Annotated *Drosophila* genome Complete gene candidate CG18434 -couch potato RNA binding protein

Human homologue of Complete gene candidate 2224621 dbj|BAA20798| (AB002338) KIAA0340

[Homo sapiens] (2e-19) and Ensembl predicted peptide Gene:ENSG00000070877

Clone:AC009710

Contig:AC009710.00004 (predicted unknown protein)

Putative function Possible RNA binding protein

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Example 36 (Category 4)

Line ID

238/37

Category

Meiotic defects in testis: segregation defects, multi-stage defects

(P1-02/17)

Reversion

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?

Map Position

70D

Rescue ID

I7E

10 Rescue Sequence

Genomic hit, Accession No. CSC:AC017664

25 Associated ORF

Genscan ORF1 predicted sequences >15:26:30 GENSCAN predicted_peptide_1 | 1819_aa EMVOAKDPPSHYLSKLRTYLDPKASRSHRLYLFYFLCQKRKMVGESTSTQVLRD LEISLRTNHIEWVKEFLDDTNOGLDALVDYLSFRLOMMRHEQRLQGVLCASEERL NLTNGGDGGEIVMGNSSSVSPGGGGGLLSHGNSTGHGLANGTLDSRQQHTMSYG FLRPTIADALDSPSLKRRSRHIAKLNMGAATDDIHVSIMCLRAIMNNKYGFNMVIQ 30 HREAINCIALSLIHKSLRTKALVLELLAAICLVKGGHEIILGSFDNFKDVCQEKRRF **QTLMEYFMNFEAFNIDFMVACMQFMNIVVHSVEDMNYRVHLQYEFTALGLDKY** LERIRLTESEELKVOISAYLDNVFDVAALMEDSETKTSALERVQELEDQLEREIDR NSEFLYKYAELESESLTLKTEREQLAMIRQKLEEELTVMQRMLQHNEQELKKRDT LLHTKNMELQTLSRSLPRSASSGDGSLANGGLMAGSTSGAASLTLPPPPPPMPASP35 TASSAAPPPPPPPAPPAPPPPPGFSPLGSPSGSLASTAPSPPHAPPMLSSFQPPPPPVA GFMPAPDGAMTIKRKVPTKYKLPTLNWIALKPNQVRGTIFNELDDEKIFKQIDFNE FEERFKIGIGGALRNGSNGTEVDGSLOSSKRFKRPDNVSLLEHTRLRNIAISRRKLG MPIDDVIAAIHSLDLKKLSLENVELLQKMVPTDAEVKSYKEYIIERKDQQLLTEED KFMLOLSRVERISSKLAIMNYMGNFVDSVHLISPQVQSIAGASTSLKQSRKFKAVL 40 EIVLAFGNYLNSNKRGPAYGFKLQSLDTLIDTKSTDKRSSLLHYIVATIRAKFPELL NFESELYGTDKAASVALENVVADVQELEKGMDLVRKEAELRVKGAQTHILRDFL NNSEDKLKKIKSDLRHAQEAFKECVEYFGDSSRNADAAAFFALIVRFTRAFKQHD

45 KKLLQQDEVYNGALEDILLGLKSEPYRRADAVRRSQRRRIDNNRLSRTLEEMDCL HENDLVKCALIADVLNLRSVHVTPVSSKDWEIIELSTEKISGSVLEQTRIVNSTQILI

OENEORLRLEKAAALAASKKENDOVLMRNKVNQKKQQEAVINELKSKAHSVRE

133

VWINKSMQVALTVDRLKPHMNYGRIDHNTELVVAPNLYKGLTNGTSNGVIEENT KLSRSKTTAQVKDELTEKLTPLTHSSTVSNVKNTIQRNKRQDHMERLKKDLRRES SRSFEFRVIRGLWREQAQESDVFVNGKHLPEFFDLDLFYCMHTAADKDYYVRVR TVEDDIEDDLPETIHPSIELNANLMKLLGIKELERVVLRPKTTVVNFVEKIELFANK 5 KTHYKIMENAFKRFVIERTQHKPMLFNQEEVVRLEDDLLVTVGILPEHFRYCVVD AQFLKESKIYAADLVRPVGEIIKEETPPTSPLSVQDLIQLPEYDKIVDQVVQELRMN LCLSADNSVMRQCNVLLAGASGTGKTVLVERILDQLSRKPDYCHFEFFHGSRSKG RKTESIQKDLRNIFTSCLQHAPAIVVLENLDVLAHAAGEQSSQDGEYYNRMADTV YQLIVQYTTNNAIAVIATVNELQTLNKRLSSPRGRHVFQTVARLPNLERADREIILR 10 ELCSHINVAKDLDLVKFSNLTEGYRKCDLVQFVERAIFYAYRISKTQPLLTNDQLI ESLEHTNSYCLQGIQSNQRTGNDADANEMRVEELPGLESVVGVLEEVLMWPSRY PTIFNASPLRNQAGVLLYGPPGTGKTYLVSQLATSWNLRIISVKGPELLAKYIGQSE ENVRNLFNRARSARPCVLFFDEFDSLAPKRGHDSTGVTDRV

15 >15:26:30|GENSCAN_predicted_CDS_1|5457_bp

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gaa at ggt gcag gcaa ag gat ccgccct cacattact t gag taa act gcgcacat at ct ggacccaa ag gcat caa ggag tcat cacattact t gag taa act gcgcacat at ct ggacccaa ag gcat caa ggag tcat cacattact t gag taa act gcgcacat at ct ggacccaa ag gcat caa ggag tcat cacattact t gag taa act gcgcacat at ct ggacccaa ag gcat caa ggag tcat caa ggag tcat cacattact t gag taa act gcgcacat at ct ggacccaa ag gcat caa ggag tcat cacattact t gag taa act gcgcacat at ct ggacccaa ag gcat caa ggag tcat cacattact ggacccaa ag gcat cacat act gcgcacat at ct ggacccaa ag gcat cacat act gcgcacat at ct ggacccaa ag gcat cacat act gcgcacat agetgegeaegaaecacategagtgggtgaaggagtteetggatgacaegaaecagggtetggaegecetggtegaetateteag cttccgactgcagatgatgcgacacgagcagcgccttcagggtgtcttgtgtgcctcggaggagcgtctgaatctcacaaacggc gggacatggtctggccaatggcacacttgactcgaggcagcagcacacaatgtcctatggattcctacgacctaccattgccgat cattatgtgcctgcgagctatcatgaacaataagtatgggttcaacatggttatccagcatcgcgaggccatcaactgcattgccttg ggcett taacatag att ttat ggtt gcet gcat gcagt tcat gaacat cgtt gtccact cggt ggag gacat gaact ac ag ggt gcacact gaacat gaact ac ag ggt gcacact gaacat gaacat gaact ac ag ggt gcacact gaacat gatta cag tacg ag ttta cag ceet gg gett gg at aag ta tet gg ag eg aat teg at tg acag aat teg ag gg aact ga ag gt ge ag at teg at tacg ag teg ag ga act ga ag teg aggettgaggatcaacttgagegagaaatagategtaacteagagtteetetataagtatgeggaattagagteegagagtetaaeget acgagcaggagctgaagaaacgggacacactgctgcacacaaagaacatggagctgcagacgctttcgcgttccctgccacga tecgectecageggegatggttetetggegaatggtggecteatggetggttecacategggggeagectetetaaeattgecaceacteggegatggttecacategggggeagectetetaaeattgecaceactegggggeagectetetaaeattgecaceactegggggeagectetetaaeattgecaceactegggggeagectetetaaeattgecaceactegggggeagectetetaaeattgecaceactegggggatggttecacategggggatggttecacategggggatggttecacateggatggttecacategggggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateggatggttecacateacctcogccgccaatgcccgcctactgcaagttcagctgctcctccaccacctccgccgccagcaccaccggctccaccaccaccg ccgg gct tcag tccg ctgg gcag tccg ag cgc acat gcctcg acat gccccg ccacat gccccg cccc acat gccccg gccacat gccccc gccacat gccccc gccacat gccccc gccacat gcccc gccacat gcccc gccacat gccccc gccacat gcccc gccacat gccaatgctaagctccttccaaccgccaccgcctccagtggccggctttatgcccgctcccgatggcgccatgaccatcaaacgcaagg acgaaa agatctt caagcaaatcgactt caatgagtttg aggagcgctt caagatcgggattggcggtgctttgcgcaatggtagcgttaagaaacattgcaatctcccgtcgcaagctgggtatgcccattgatgatgtcatcgccgccattcatagtctggacctgaagaa egcaaggaccaacagctactcaccgaagaagacaagtttatgctgcagttgtcgcgtgtggagcgtatctcgtccaagctagcca ttatgaactatatgggcaattttgtcgacagcgttcatctcattagtccgcaagtgcaatcgatagcaggagcgtcgacttccttaaaa etttaagetgeaategetggacaegetgategataeaaaateeacagaeaagegategteactgetteactatattgtggeeaceat ggtggccgatgttcaggagcttgaaaagggcatggatctggtgcgcaaggaggccgagctgcgagtgaagggtgcccagacg

teaggtgettatgegeaacaaggttaaccagaagaagcaacaggaagctgtcataaacgagctgaagagcaaggcgcactegg gtacaggcgggcggatgctgtgcggcggtcgcagcgcggaggatcgacaataatcgtttatcgcgcaccctggaggaaatgg 5 attgtctgcacgagaatgatctggtcaagtgtgcgctcatcgctgacgttctcaacctgcgcagcgtccacgttacccccgtctcgtccaaggactgggagatcatagaacttagcactgaaaagatatcgggcagtgtgctggaacaaactcgcatagtgaattcaacgca gateettattgtttggattaataagtegatgeaagttgegetgacagtggategtetgaageegeacatgaactaegggagaataga tcacaatacggaactcgtggtggcgcccaatctgtacaagggtctgaccaatggaacttcaaatggtgttatagaggaaaacacaa 10 gettegaatttegtgtcattegaggtetatggegggageaggeecaggagteggatgtgtttgtgaaeggaaageatetgeetgag ttetttgatetagatetattetattgeatgeaeaeegeageegaeaaggattaetatgtgagagtgegeaeagtagaagaegatattg aggacgatctaccagaaaccattcatccatcgatcgaactaaatgccaatcttatgaagttgctgggtattaaggaattggaacgag tggttctaagacctaaaactaccgtagttaactttgtagaaaaaattgagctatttgccaacaagaagacgcactacaaaatcatgga 15 gaacgcatttaagcgatttgtgatagagagaactcagcacaagccgatgctcttcaaccaggaggaggtggtacggctggagga cgatttactggttactgttggaattttaccagaacactttcgttattgcgtggtggacgcgcagtttctgaaggagtccaagatctacg cagtgcaatgtcctactcgctggtgcctcgggaacgggtaaaacagttcttgtggagcgcattttggaccagctgtcacgcaagcc 20 taccagctgcctgcagcatgccccgccattgttgtgctagaaaacttggatgtactggcccacgctgctggagagcagtccagtc aggatggagagtactacaatcgcatggcggatactgtgtatcagttgattgttcagtataccaccaacaacgctattgcagtaatcgaatttggaacgagcagatcgagagataattcttcgagagctgtgcagccatatcaatgtggccaaggacctggatcttgttaagttct 25 agcetettet gacea at gatea gettatt gagtee et ggagea cacaa acteg tact geet geagggeatte agagea at caa agaal acte get geagggeatte agagea at caa agaal acte geagggeatte agagea at caa agaal acte geagggeat geagggeat gagea gagea acte grant gatea gagea acte grant gatea gagea acte grant gatea gagea acte grant gatea gagea gagea acte grant gatea gagea gageaactgg caatgatgccgatgccaatgaaatgcgcgtcgaggagttgcctggcctggagtcagttgtgggagttctggaggaggtccttatgtggccatcaaggtatccaaccatttttaacgcctctccactgcgcaaccaggccggagtacttctatatgggccaccaggaacagg taaaacctatet gg tetete agt t gg cacate g t gg aac et ge ge at catt t ceg tea ag gg te et gag t t ge te ge aaat a te ge ge at catt t ceg te a gg te et gag t t ge te ge aaat a te ge ge at catt t ceg te a gg te et gag t t ge te ge aat catt t ceg t ca ag gg t cet gag t t ge te ge aat catt t ceg t ca ag gg t cet gag t t ge te ge aat catt t ceg t ca ag gg t cet gag t t ge t ceg ag t ge at cat t ceg t ca ag gg t cet gag t t ge t ceg aat cat t ceg t ca ag gg t cet gag t t ge t ceg aat cat t ceg t ca ag gg t cet gag t t ge t ceg aat cat t ceg t ca ag gg t cet gag t t ge t ceg aat cat t ceg t ca ag gg t cet gag t t ge t ceg aat cat t ceg t ca ag gg t cet gag t t ge t ceg aat cat t ceg t ca ag gg t cet gag t cet g cet gag t cet gag t cet gag t cet gag t cet g cet g30 agcttggcgccgaaacgtggtcacgattccacgggggtcaccgatcgagtg

Drosophila Gene Hit recue sequence and TBLastn with ORF1: mRNA for l(3)70Da (AJ243811)

35 Human Homologue

Human Homologue BLASTX with 1(3)70Da: peroxisome biogenesis factor 1

(AF026086)

Drosophila EST LD43687 (AI512050)

40 Annotated *Drosophila* genome genomic segment AE003536

Annotated Drosophila genome Complete gene candidate CG6760 mRNA for l(3)70Da

- novel protein with homology to endoplasmic reticulum ATPases

4505725

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ref|NP_000457.1|pPEX1| peroxisome biogenesis factor 1>gi|2655141 (AF026086) (8e-80)

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Putative function

Putative member of the AAA protein family (ATPases associated with diverse cellular activities) including homologies to transitional endoplasmic reticulum atpases, and an E.coli membrane-bound AAA-type metalloprotease which degrades degrades sigma32, an alternative sigma factor for heat shock promoters

15 **Confirmation by RNAi** G2/M

Slight loss of G1, increase in G2/M indicating arrest in

136

Line ID

238/44

Category

Meiotic defects in testis: segregation defects, multi-stage defects

(P1-02/18)

Reversion

Ŕ

5 Map Position

70D

Rescue ID

F8E

Rescue Sequence

GTTCAAACGCACTTTTAAGGTGGCCTATCGGCCCATCAGGAGCAACTTTGTTC

10 TGCTGCCGGATCAGTACTACGGCGTCGTTTCGACCTATGTAAGTGTCTAAAGG
 TCTTCGCTCCATTCAGATTAGATACGCCAAAGATTAATCCGGTCAACCATTCT
 GATTAGGACACGGGCTGCCTGAGCTTGCAGTACAATGGTCGGACGCACTACG
 CCTCCTGGGCGCCACAAAAAGGGCGGCGGCGGCATTAAAGACACCGAGATTGG
 GATCAATGCCAGAGCGCCAAGGAGATCGGTAAGCCATTACTTAACGGCCGG

15 ATGTGCATCGGTTGCCAATGTGCCGTAATATTGGACTCCGGCCATTTGCCCCG
 TACCTCGTACGCTAGCAGCACCCACTTACCCTTTCTTGCCGTATGTCTGCACGA
 GAATGATCTGGTCAAAGTGTGCGCT

Other results same as for line 238/37

20

Line ID

428/5

Category

Meiotic defects in testis: cytokinesis defects, segregation defects

(seg-01/01)

25 Reversion

?

Map Position

70A

Rescue ID

G4E

Rescue Sequence

- 30 GTTCAAACGCACTTTTAAGGTGGCCTATCGGCCCATCAGGGAGCAACTTTGTT
 CTGCTGCCGGATCAGTACTACGGCGTCGTTTCGACCTATGTAAGTGTCTAAAG
 GTCTTCGCTCCATTCAGATTAGATACGCCAAAGATTAATCCGGTCAACCATTC
 TGATTAGGACACGGGCTGCCTGAGCTTGCAGTACAATGGTCGGACGCACTACG
 CCTCCTGGGCGCCACAAAAGGGCGGCGGCGGCATTAAAGACACCGAGATTGG
 35 GATCAATGCCAGAGCGGCCAAGGAGATCGGTAAGCCATTACTTAACGGCCGG
 ATGTGCATCGGTTGCCAATGTGCCGTAATATTGGACTCCGGCCATCTGCCCCG
 TACCTCGTACCTAGCAGCACCCACTTACCCTTTCTTGCCGTAGGTCTGCACGAA
 AATGATCTGGTCAAGTGTGCGCTCATCGCTGACATTCTCAACCTGCGCA
- 40 Other results same as for line 238/37

137

Line ID 848/7

Category Mitotic defects in brain: cytokinesis defect. Meiotic defects in

testis: cytokinesis defect. Multi-stage defects

Polyploidy, no overcondensation

Pl-01/10

Reversion R **Map Position** 70D1-2

Rescue ID G1E

10 Rescue Sequence 1

5

GGCCACCTTAAAAGTGCGTTTGAACATTCTCGTCGTGGGCGTGTGCGAATTTA GTACGCTCCTTGCTTTAAATCATTTTCGCACTAAACTTCTGCTCTCAGCGG AATTTACTTTTGCTTTATTAGAGATGGGAGCTCGCGCATCAGCTGAGCCGATA CTTGCGCAACAGGTGATACAGCTGATTAGAGATGGCCCTTTTCAACTGTTCCC

20 ATATCGATTTCCCTTCACTTTCGCTCCTCGTATACCATGCTGGGGTCTTATCAA ATTTATT

Rescue ID G1P

Rescue Sequence 2

25 AAGGTGGCCTATCGGCCCATCAGGAAGCAACTTTGTTCTGCTGCCGGATCAGT
ACTACGGCGTCGTTTCGACCTATGTAAGTGTCTAAAGGTCTTCGCTCCATTCAA
ATTAGATACGCCAAAGATTAATCCGGTCAACCATTCTGATTAGGACACGGGCT
GCCTGAGCTTGCAGTACAATGGTCGGACGCACTACGCCTCCTGGGCGCCACAA
AAGGGCGGCGGCGCATTAAAGACACCGAGATTGGGATCAATGCCACAGCGG
30 CCAAGGAGATCGGTAAGCCATTACTTAACGGCCGGATGTGCATCGGTTGCCAA
TGTGCCGTAATATTGGACTCCGGCCATCTGCCCCGTACCTCGTACGCTAGCAG
CACCCACTTACCCTTTCTTGCCGTAGGTCTGCACAGCGTCCACGTTACCCCCGT

CTCGTCCAA

Other results same as for line 238/37

138

Example 37 (Category 4)

Line ID 252/40

Category Meiotic defects in testis: segregation defects, abnormal spindles.

(Ab-03/30)

Reversion R **Map Position** 84E

Rescue ID A4B

10 Rescue Sequence 1

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TACATGACTCTGCGATTTGACAAAAACAAAATTGAGTTTTGTCAAGAAAATCA
ACTATTTTCTGTGTTTAAAAAACCGAAGCCAACAAATCCGACCAAAATGCCT
GCCGAAAACTTGGAGGAGCAGGGTCTGGAGAAGAACCCGAACCTGGAGCTGG
CCCAGACGAAGTTCCTGCTTACCCTGGCGGAATACAAGCAGGATGCGGCATTG
AAGGCGAAGCTTCTGGAGGCGATTCGCACGGAGAATATGGCCCCGTGGGTAC
GAGCACATCCTGCTCCGGAACTCGGCTTGGACCCGTTAGACAAGGATCTTGCC
TGGCGCCGAATTGAAGGAAAAACAATCGCGTTTAAGTTGGGAGCCA

Rescue ID A4E

20 Rescue Sequence 2

- 30 ATTGATTTCCTGATAAAAATTTTCGCTTGGAAGCTACAGCATCGTCCACTGTC CATGTTTATATATCCTTATATTTGCCTATAAATATAT

Genomic hit, Accession No. AC006494

35 Associated ORF

Genscan: ORF1 predicted sequences >23:00:28|GENSCAN_predicted_peptide_2|389_aa MPAENLEEQGLEKNPNLELAQTKFLLTLAEYKQDAALKAKLLEAIRTENMAPWY EHICSELGWTVDKDLLARMKENNRVEVEQLDAAIEDAEKNLGEMEVREANLKKS EYLCRIGDKAAAETAFRKTYEKTVSLGHRLDIVFHLIRLGLFYLDHDLITRNIDKA

40 KYLIEEGGDWDRRNRLKVYQGVYSVAVRDFKAAATFFLDTVSTFTSYELMDYPT FVRYTVYVAMIALPRNELRDKVIKGSEIQEVLHGLPDVKQFLFSLYNCQYENFYV HLAGVEKQLRLDYLIHPHYRYYVREMRILGYTQLLESYRSLTLQYMAESFGVTVE YIDQELARFIAAGRLHAKVDRVGGIVETNRPDNKNWQYQATIKQGDLLLNRIQKL SRVINI

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>23:00:28|GENSCAN predicted CDS 2|1170 bp

139

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Drosophila Gene Hit BLASTN with rescue sequence 1 and TBLASTN with ORF1: 26S

proteasome regulatory complex subunit p42A (AF145308).

Human Homologue BLASTX with EST and TBLASTN with ORF1: Hypothetical

protein KIAA0107 (D14663).

20 Drosophila EST

several including GH17651 (AI387197)

Annotated *Drosophila* genome genomic segment

AE003739

Annotated Drosophila genome Complete gene candidate CG5378 - Rpn7 19S

25 prote

proteasome regulatory

particle, non-ATPase protein,

subunit S10aHuman

Homologue

30 Human homologue of Complete gene candidate

gi7661914

8843E6684AE91ACD

|ref|NP_055629.1| KIAA0107

gene product [Homo sapiens]

(3.40E-149)

35

Putative function component of the 19S proteasome regulatory particle

Confirmation by RNAi Marked decrease in G1 and G2/M indicating fewer cycling cells

40

140

Example 38 (Category 4)

Line ID

277/7

Category

Mitotic defects in brain: anaphase defects

(weak, higher condensation, some polyploidy, fewer anaphases,

polyploids with monopolar spindles)

Reversion

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7

Map Position

71B

Rescue ID

B8E

10 Rescue Sequence

20 TGCAAAGACCACAAACAAATCATTAGGGGCGT

Annotated *Drosophila* genome genomic segment AE003584 Annotated *Drosophila* genome Complete gene candidate CG15383 – novel

25 Human homologue of Complete gene candidate none

Putative function

No homologies to indicate function

Confirmation by RNAi

Slightly increased G1 decreased G2/M indicating arrst in G1

30

PCT/GB01/01297

141

Example 39 (Category 4)

Line ID

284/4

Category

WO 01/72774

Mitotic defects in brain: anaphase defects

(overcondensation, polyplody (with overcondensation), few

anaphases, metaphase with bipolar spindle)

Meiotic

Reversion Map Position NR

89B

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Rescue ID 2C6E

Rescue Sequence

GTCTACCACTAGCTCTTTGTCTTCGCCTTCTAGTCTCTCATCTTGGCAGCCC GTTCTAGTGCGCGTATTTTTAGTCGCAACACATTGCCCAATTCGCCAGCCGCTA 15 CATTTAACAATAATCCCTGCGTTCGCTGTCCACGTCCACATTACGATACGTTTA GTGCACGGAAAGAATAAGCGTGTGGTTTCATAATATTAGCTATTGAAAAAA GTTCTTAAATTTAAGCCTCACTCGATTCTGATGCATGAAATATTATTGGATTGT AAATGAGCGTCATGTTTTGGTATACAAATCTCAAAGTAATTTAAAAATTCTCA TCTTACCGTACCTTGAACCACTACCAATCATCTCAGTACAGCATTTCAGCGAA 20

CAACTTGGGGTAGGGCACCTGAACTAGTTTCAAACGGCGGCGGTCGGCCTTTT CAGCTTTTTCGCATTTGCCATTTTCCCGCGG

AE003711

Annotated Drosophila genome genomic segment Annotated Drosophila genome Complete gene candidate CG4275 - mor transcription

factor involved in chromatin

remodelling

30

25

Human homologue of Complete gene candidate

CG4275- 4507081

|ref|NP 003066.1|pSMARCC 2 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2(aa)

35

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Transcription factor, regulator of chromatin

Confirmation by RNAi

Putative function

Decrease in G1 and G2/M and increase in polyploidy

142

Example 40 (Category 4)

Line ID 407/8

Category Meiotic defects in testis: cytokinesis defects

5 Reversion ?

Map Position 64B1-2

Rescue ID A9E

Rescue Sequence

Genomic hit, Accession No. AC005814 64A6-64B6

Associated ORF

- Genscan ORF1 predicted sequences >22:57:22|GENSCAN_predicted_peptide_2|524_aa MGRRKDKPRVIPEQDARICRAICLCQLTMVLSCVSIVYLSVAIYSPSLKAFKSGFEL DPVMCQTVDRQMPNNCPWASCGEWCLTKTSGFCPQIHSIVRRNGTDIQLNNCTR VTNTSCAMIDLSRLNKFNCNNGTACNNIRGVFNCSNGHCKNMSEFFLCHHKADG LTVNSQKDNTKLNGFFECHGVHCTKIKKPFSCDRYCSKITTTNVNTLIMHEDNLIA
 ADCENAVAFNQARGSEHGVRIEPFEFWKEDDGNLLTNCATVTRESDNRITATDCI NGTLLEHDTLPAPFMNFTQFWAIYENSTRSVDPEQRYLPNQANLTIYSWKKLFINL EGCVNTLRGECKDFVARYGNDGDNNTAQSRYQCYYNKDSNVEFVVARYDLDK VYRELLVSLIVPIVLFVISSISLCIITKSVKVGDDAKMRCVCAGDDSDNDGPFGPGL ANKQQDQMYDTDDDVVDLEHQAVDGQELSDHGLPLDNQELIGSTKSLIPISPVGE
- 35 SGTSDQIFDQDQEKATTCDVPEKPLVIL

>22:57:22|GENSCAN predicted CDS_2|1575_bp

143

(corresponds to CG15003)

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Annotated *Drosophila* genome genomic segment AE003480
Annotated *Drosophila* genome Complete gene candidate CG15003- novel unknown

Human homologue of Complete gene candidate none

20 **Putative function** No homologies to suggest function

Confirmation by RNAi Only wild type profiles observed

144

Example 41 (Category 4)

Line ID 422/28

Category Meiotic defects in testis: segregation defects, multipolar spindles

(Mul-02/22)

Reversion NR **Map Position** 68E

Rescue ID 2I4E

10 Rescue Sequence

TCGTGGACCCTCAAAGNAACGGATTTCTCCAGTTTCTTCAAAGGGTTAATAAA
CTTTTCGCACGTTTCGCATTTTTATGCTCAATCCGGTTACAAAATGCTGATAAA
ACCACTTGAACTACACGGTTTCCGTACTGATAAGGGCTTTTCTTCTTATCTGACC
TCTGGAATTCCGCGGAATTAATTCTTGAAGACGAAAGGGCCTCGTGATACGCC

15 TATTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCA
CTTTTCGGGGAAATGTGCGCGGGAACCCCTATTTGTTTATTTTTCTAAATACATT
CAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATAT
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTT
TTTGCGGCATTTTGCCTTCCTGTTTTTTGCTCACCCAGAAACGCTGGTGAAAGTA
20 AAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCT
CAACAGCGGTAAGATCCTTGAGAGTTTTCCCCCGAA

Genomic hit, Accession No. CSC:AC014962

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Annotated *Drosophila* genome genomic segment AE003543

Annotated *Drosophila* genome Complete gene candidate CG5684 (putative

transcription factor, human

homolog

30

Human homologue of Complete gene candidate 1e-100 4758946

ref|NP_004770.1|pPOP2| POP2 (yeast homolog) >gi|4106061|gb|AAD02685| (AF053318) CCR4-associated regulator of polymerase II

transcription

40

35

Putative function Transcription factor

145

Example 42 (Category 4)

Line ID

WO 01/72774

422/5

Category

Meiotic defects in testis: segregation defects, abnormal spindles

PCT/GB01/01297

(Ab-04/26)

Reversion

5

?

Map Position

82D

Rescue ID

B9E

10 Rescue Sequence 1

Rescue ID B9B

Rescue Sequence 2

Genomic hit, Accession No. AC008189

Associated ORF

Genscan ORF1 predicted sequences >15:53:24|GENSCAN_predicted_peptide_3|211_aa
40 MRNANESSGKPKSKFVSNEFHALFSTICSIADSPAVSREKLKIDLAARKIPSASAPK
GDSPLERFSRDLFTYLRSVCRWGRFSAALFTAELLIVGGIVSSNRTSESSETGNPLA
NEPDPLYMKLVDPMVAGESPKRMIKDQKDVGLKSTSSSEELRKLPKTRGRQKRFI
RNPNYVKANEFYDKMLSSEYVSKRYKDLPPPHPGFGADQPPA

45 >15:53:24|GENSCAN_predicted_CDS_3|636_bp atgegeaaegeaatgaategagegtaaaccaaaategaaattgtaageaaegaattcaaegaattgtttcaacaatttgttcaa

146

Corresponds to CG2503

10

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Annotated *Drosophila* genome genomic segment AE003605

Annotated *Drosophila* genome Complete gene candidate CG2503 - novel possibly RNA binding

15 Human homologue of Complete gene candidate

3287674 AC005239 (AC005239) F23149_1(aa)

Putative function Possible RNA binding protein

20

Confirmation by RNAi Almost no G1 and broadened G2/M indicating arrest in G2/M

147

Example 43 (Category 4)

Line ID

423/14

Category

Meiotic defects in testis: cytokinesis defects, abnormal spindles

(Ab-16/13)

Reversion

5

15

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Map Position

67B1-10

Rescue ID

E9E

10 Rescue Sequence

20 CAATAATTGTAATTTATCCTTACAAAATGTTA

Genomic hit, Accession No. CSC:AC020214

Drosophila EST

several including LP12306 (AI297868)

25 Annotated *Drosophila* genome genomic segment AE003552

Annotated Drosophila genome Complete gene candidate CG3967 - novel

Human homologue of Complete gene candidate

none

30 Putative function

No homologies to indicate function

Confirmation by RNAi

Only wild type profiles observed

148

Example 44 (Category 4)

Line ID 427/5

Category Mitotic defects in brain: anaphase defects. Meiotic defects in testis:

segregation defects, abnormal spindles

(mitotic: Overcondensation, lagging chromosomes/less aligned

metaphase with bipolar spindles, Meiotic: Ab-06/20)

Reversion ?

Map Position 67B1-5

10 Rescue ID H4E

Rescue Sequence

5

GTACAGCCTGAAGTGATCGTTGTTTGTTTGAATCGGTGCTATCGGCGGTTGCGC
TTTGTGGGCATCTTTATCCAATTTGCTATGCGCGCTTGTCCTTAAATTTTGAAC
TGTATTCCAAGGGTTGCTTTGGCGGCTATCGATAGTATCGGCATGGTTACATTT

15 TAGTTTTATAACAAGAATTTTACAGGTATTTTGATTATCTGAGCTTAGTTTTAA
GCAANAATATTATTGTTAAAAAATTTAAAAAAGTAAACAAGCTATTTTAACAAGC
ATTTAAACAAATAGTATTAATAATATAAAAAATATTCGATATGTGTTGCAAAT
GTTCGTTCCCTTAGTATTCTCTCATATTTATTTCAAATAAACTGTATAAAAATAT
CTGAAAAAAGCGAACATATTTATTTAATTTCATCGCAGATATCGATATCACAGC

20 GCTGCTATCGATGGTGTCTCTCGCAGTGCCTATCGCTTACCCTGCCATCGCT
AACAAAAA

Genomic hit, Accession No. CSC:AC020120

25 Associated ORF

Genscan: ORF2 predicted sequences >22:06:07|GENSCAN_predicted_peptide_7|464_aa
MPSEQHTNIKVAVRVRPYNVRELEQKQRSIIKVMDRSALLFDPDEEDDEFFFQGA
KQPYRDITKRMNKKLTMEFDRVFDIDNSNQDLFEECTAPLVDAVLNGYNCSVFV
YGATGAGKTFTMLGSEAHPGLTYLTMQDLFDKIQAQSDVRKFDVGVSYLEVYNE
HVMNLLTKSGPLKLREDNNGVVVSGLCLTPIYSAEELLRMLMLGNSHRTQHPTD
ANAESSRSHAIFQVHIRITERKTDTKRTVKLSMIDLAGSERAASTKGIGVRFKEGAS
INKSLLALGNCINKLADGLKHIPYRDSNLTRILKDSLGGNCRTLMVANVSMSSLTY
EDTYNTLKYASRAKKIRTTLKQNVLKSKMPTEFYVKKIDEVVAENERLKERNKA
LEAKATQLERAGNSGFDPLELKTWYSKIDAVYAAARQLQEHVLGMRSKIKNINY
RQTLKKELEEFRKLMCVDQRVCQESF

>22:06:07|GENSCAN predicted CDS_7|1395_bp

atgcetteggaacagcatacgaatataaaagtggcggttegegtacggcgtataatgteegtgaattggagcaaaaacagegga
gtattateaaggteatggategtteggeactgetgttegateeegaegaggaggaegatgagttettettteagggegeeaageaac

40 egtacegegacateaceaageggatgaacaaaaagttgaceatggaattegacagggtattegatatagacaatteeaaceagga
tetgttegaggagtgeaeggegegetggtegaegeggtgttaaatggatacaactgeteggtatttgatatggagecaetggeg
eeggaaaaacatteacaatgetgggeagegaggeteateegggtetgacetatettaceatgeaagatetettegataagateeaa
gegeagagegaegtgegeaagttegatgtgggggtateetatetagaggtgtacaacgaacatgtgatgataatetgetaactaaate
gggecetttaaaacttegegaggacaacaatggegtggtggteagtggtetttgeteaegeeaatetacagtgeegaggagetge

45 taagaatgetgatgetgggeaacteteategaacteagcacceaaaagaacggteaaatgeagagtteeaggteacatgeegaggagtga
gagggegecagtacgaaaggegeaagacgaacaccaaaagaacggteaaactateeatgategatetggegggaattga
gagggeggecagtacgaaaggegttggagtgegatteaaggaaggegecagcacaaaaaagtetettagetttgggaaattg

149

cataaacaagetageegaeggettaaageacateeegtaceggaetegaacetgaacetgaacegcateetgaaggaetegttgggegg aaattgtegeacattgatggtggeeaatgtetegatgageteactgacetatgaagatacetacaacaceettaagtaegetageeg agetaagaagatacgcaegaetetgaaacagaatgteeteaagteeaagatgeeaacegagttetatgtgaagaagategaegag gtggtageegagaacgaggegaacaaagagegeaacaaggegetggaggeeaaggeeacteagttggagegegeggeaat agtggattegateegetggagettaagaegtggtaeageaagatagaegetgtatatgeggeegeeggeagetteaggageac gteettggtatgegtageaagateaagaacateaactaaceggeagaacatgaaaaaagaactggaggagtteaggaagetgatgtgttggaeeaggaggtgtgeeaggaggtttttaa

Drosophila Gene Hit TBLASTN with ORF2: kinesin like protein 67a (U89264) **Human Homologue** (AF041853)

TBLASTN with ORF2: kinesin family member protein KIF3A

Drosophila EST GH22018 (AI402731)

Annotated *Drosophila* genome genomic segment AE003552
Annotated *Drosophila* genome Complete gene candidate CG10923 Klp67a -

motor protein

Human homologue of Complete gene candidate	2e-58 4758646 kinesin family
	protein 3B
	>gi 3913958 sp O15066 KF3B
	HUMAN KINESIN-LIKE
	PROTEIN KIF3B and also
	predicted peptide
	ENSP00000166696
	Gene:ENSG00000073652
	Clone:AC015936
	Contig:AC015936.00023
	6.70E-91 (predicted kinesin?:

ENST00000166696)

30

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Putative function motor protein involved in cytoskeleton organization and biogenesis

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Confirmation by RNAi Almost no G1 and broadened G2/M indicating arrest in G2/M

150

Example 45 (Category 4)

Line ID

442/3

Category

Meiotic defects in testis: segregation defects.

5 Reversion

?

Map Position

70D4-7

Rescue ID

H7E

Rescue Sequence

GCTAGCTTTGAAGCATCTCAAAGGAAAAAGGGCGATAATTCTGATTA

20 Genomic hit, Accession No. CSC:AC017664

Drosophila EST

CK02287 (AA141680)

Annotated Drosophila genome genomic segment

AE003536

25 Annotated *Drosophila* genome Complete gene candidate CG6650 - novel transacylase like

Human homologue of Complete gene candidate

none

30 Putative function

Transacylase

Confirmation by RNAi

Marked increase in G1 indicating arrest in G1

151

Line ID

473/22

Category

Meiotic defects in testis: no division

(no meiosis)

Reversion

R

Map Position

70A1-5

Rescue ID

2B7E

Rescue Sequence 1

20

5

Genomic hit, Accession No.

CSC:AC017664

Drosophila EST

LD47104 (AI515336), SD03663 (AI532240)

For other results see line 442/3

25

Line ID

670/6

Category

Meiotic defects in testis: segregation defects, abnormal spindles

(Ab-12/48)

30 Reversion

?

Map Position

70C

Rescue ID

H7E

Rescue Sequence

- 45 **Genomic hit, Accession No.** CSC:AC017664 *Drosophila* EST CK02287 (AA141680)

For other results see line 442/3

152

Example 46 (Category 4)

Line ID 460/20

Category Meiotic defects in testis: segregation defects, multipolar spindles

(mitotic: High polyploids, no diploids, higher mitotic index

Meiotic: Mul-02/59)

Reversion NR **Map Position** 78A1-4

10 **Rescue ID** 2B8E

Rescue Sequence

25

35

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Genomic hit, Accession No. CSC:AC020460

Annotated *Drosophila* genome genomic segment AE003592

Annotated Drosophila genome Complete gene candidate CG10588 - novel gene with

30 homology to proteases

Human homologue of Complete gene candidate 2e-74 4505453

ref|NP_002516.1|pNRD1| nardilysin (N-arginine dibasic

convertase)

>gi|2462488|emb|CAA6369

Putative function Novel protease

Confirmation by RNAi Marked increase in G1 indicating arrest in G1

153

Example 47 (Category 4)

Line ID 477/16

Category Meiotic defects in testis: segregation defect.

5 Reversion NR? Map Position 90C5-10

Rescue ID C3E

Rescue Sequence 1

20

Rescue ID C3P

Rescue Sequence 2

35

Genomic hit, Accession No. AC007810

Associated ORF

Genscan ORF1 predicted sequences >17:48:58|GENSCAN_predicted_peptide_2|349_aa

MSRILFILLLLIVTQLSELQAAAFSVRQNRFDEVPDLQTPAPLATSTESSKKPEKAT
SGLLKKCLPCSDGIRCVPQIQCPAHVRMESHEKPQICDLPAGKFGYCCETGQNHT
APKPETSPKERRSGFPTILSPAVLDEARRNFEHLMHGVAQIPVRRGFPDFAHGLVF
HSTAKDDLHNFAISNSAIEQVMTTQLFGKKEQVPVEDFITNNVPIKFTETPLAHHC
QPPPVCGNIRSVYRSMDGTCNNPEPQRSLWGAAGQPMERMLPPAYEDVPSASPA
AICSYIYGIASRLAPVSVVNCCTFAWQLDWTTGMASGECVCVECMPAEWRLGQC

PLLHEASSEMSRLLAKS

154

>17:48:58|GENSCAN_predicted_CDS_2|1050_bp

ttgatgaagttcctgatttgcagactcctgcacctctggccacttccactgaatcttctaagaaaacccgaaaaagctaccagtggtct 5 getgaaaaaatgeetteeetgeagegatggtataagatgegtgeeceaaateeagtgteeegeecacgttegeatggaaageeat gaaaagccccaaatttgcgatctcccggctggaaaattcggctactgctgcgagactggacagaatcacactgctcccaagccg gagaceteteceaaggagegtegateeggattteceaceattetgteaeeegeagttttggatgaggegegtegeaatttegagea cttgatgcatggagttgcgcagattccggtggccgtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggcttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggcttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggcttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggcttttccactcgacggccaaggattccggtggctttccagattttgcccatggcctggctttccactcgacggccaaggattttgccactcgacggccaaggattttgccactcgacggccaaggattttgccactcgacggccaaggattttgccactcgacgattttgccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccacactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttccactcgacgatttttcgacettcacaacttcgccatatcgaacagtgccattgaacaagtgatgaccacccagttgtttgggaagaaggaggaggtgcccg 10 tagaagatttcatcaccaacaatgtgcccatcaagttcactgagactccgctggcacaccattgccaaccgcccccagtttgcggc aatatteggtetgtttategeageatggaeggeacttgeaataateeagaaceaeagagatetetgtggggtgetgetggteaaeeg atggagggatgctgeccccgcctatgaagatgttccgtcagettctcctgctgctatatgtagttatatctatggcatcgcatctcg gtggaatgtatgccggcggagtggcgtttgggccaatgcccgttgcttcatgaggcgtcgagtgaaatgagccgcctcttggcta 15 aaagctag

Drosophila Gene Hit rescue sequence: eyelid/osa (AF053091)

Human Homologue BLASTX with eyelid: KIAA1235 protein (AB033061) Brain protein 120 (AB001895)

20 Drosophila EST

25

30

35

several including LD04852 (AA201670), LD24466

Annotated Drosophila genome genomic segment AE003718

Annotated Drosophila genome Complete gene candidate CG7467 - osa DNA binding putatively involved in DNA packaging

Human homologue of Complete gene candidate

CG7467 - 7e-25 2588991

dbj|BAA23269| (AB001895)

B120 [Homo sapiens] and

B120 [Homo sapiens] and O14497 SWI/SNF-RELATED, MATRIX-ASSOCIATED, ACTIN-

DEPENDENT REGULATOR

OF

CHROMATIN SUBFAMILY

F MEMBER 1 3e-67

40 **Putative function** transcriptional regulator

Confirmation by RNAi Only wild type profiles observed

155

Example 48 (Category 4)

Line ID

5

496/4

Category

Meiotic defects in testis: segregation defects, abnormal spindles

(meiotic: Ab-08/42)

Reversion

NR

Map Position

65E4-7

Rescue ID

2C1E

10 Rescue Sequence

GCACGATCGCTCTCTTGGCTCTCTATCACTCTCTGGACTCTCTCAGCA CCTTTGCTACCGTTTCGCAGAACAGGTGTATCGGTTTTCAAGGCAACTGTGATT TTTTAACTCAACATTCTATATCGAAAACTTGTAGAGGTCGGAATTTTTCTTGAG CGCCTAAAAGTGTGCAGTGAAATCATTAAACAAGGTTTGACACCCTTCAAATA

- 20 TTATTGTTATTGCGGTGGCTGTAGATGTAAATGTAAATGTAGATGTAGAGGCT GCTTCTTGGG

Genomic hit, Accession No. CSC:AC018039

25 Associated ORF

Genscan ORF1 predicted sequences >19:35:36|GENSCAN_predicted_peptide_6|190_aa MVSEQFNAAAEKVKSLTKRPSDDEFLQLYALFKQASVGDNDTAKPGLLDLKGKA KWEAWNKQKGKSSEAAQQEYITFVEGLVAKYDNGMHKQEPNTCQARNATRFR KSSECSLDQNTYTSSVTVIPAFHEGPKNSTASWPRIYRCYQRNQQAANCKWANTN SVCGKPHGKQSRRIIFAEFLAGHTVQILG

>19:35:36|GENSCAN predicted CDS 6|573 bp

atgettteegageaatteaaegeegeegeegagaaggtgaagageetgaceaagegteeeagtgatgaegagtteetgeagetg
taegeeetgtteaageaggeeagegttggtgacaaegacaeegeeaageegggteteetggacetgaaggeeaaggeeaagtg
ggaggeetggaacaageagaagggeaagageggaggeegeecageaggagtacateaeetttgtggagggeetggtgge
caagtatgacaatggaatgeacaaacaagaaceaaacaettgeeaageacgeaatgegaeteggttteggaaaageteggaatg
etegetggateagaataegtataegteeagtgtgaeggttataeetgeatteeaegaaggteeaaagaaetegaeggeaagttgge
caagaatttaeeggtgetateageggaaceaacaageggeeaaetgeaagtgggaaaacaaaatagegtttgegggaaacee
caeggaaaacagageegeegaateattttegeagaatttetggeeggeeataeggtgeagattettgggtaa

40

30

Drosophila Gene Hit rescue sequence: melt (S144114) P element insertion site (AF174669), TBLASTN with ORF1: diazepam binding inhibitor (DBI) (U04823) and melted (AF205831)

45 Annotated *Drosophila* genome genomic segment AE003560
Annotated *Drosophila* genome Complete gene candidate CG8624 melt - putative signal

156

5			transduction protein CG8631 msl-3 - acyl-CoA- binding protein/diazapam binding inhibitor
	Human homologue of Com	plete gene candidate	CG8624- predicted gene ENSP0000065899 Gene:ENSG0000055889
10			Clone:AC015904 Contig:AC015904.00014 1.70E-15 (unknown predicted gene 1: ENST00000065899 and AK022666 Homo sapiens cDNA FLJ12604 fis 2e-29
15			CG8631- gi5803104 0C85AE40FDF874CD
20			ref[NP_006791.1 male-specific lethal-3 (Drosophila)-like 1 [Homo sapiens] (1.70E-36) and Ensembl predicted peptide ENSP0000006617 Gene:ENSG00000005302 Clone:AC004554
25			Contig:AC004554.00001 8.70E-19 (unknown predicted gene 1: ENST00000006617
30	Putative function	CG8624: putative sig	nal transduction protein
	inhibi	CG8624: putative signal transduction protein CG8631:acyl-CoA-binding protein/diazapam binding tor	
35	Confirmation by RNAi		and G2/M Indicating fewer cycling ased G1 to G2/M ratio indicating arrest

157

Example 49 (Category 4)

Line ID

523/19

Category

5

Female sterile. Meiotic defects in testis: cytokinesis defects,

segregation defects (Mitotic: Less condensed chromosomes, nuclear

bridges, Meiotic: Seg-01/02

Reversion

R

Map Position

75C1-4

10 Rescue ID

2B4E

Rescue Sequence

- 15 AAGAATGAAGCCAATGAATTTTCAATAGTAATTCAGAGTGCTTAAAATT CTTCATGTTGTCATTGAGTAAAATGAGTTCGGACAGCGCGAAGGTAAGTCGAA GTTTGTGTTTTATTATGTTATTTGTATTATGTACACTAGTCGGCATACTTT TGCGTGCGTCTTATACGTGTGCGTCTTATTTAACAATATTGTAAAATAAAATAT ATAAATTATTTGTTATATGCGTAGGGGCCTTTATTTTGTGTATATTGATAGTCTTTT
- 25 Genomic hit, Accession No. AC007691

Annotated *Drosophila* genome genomic segment AE003520 Annotated *Drosophila* genome Complete gene candidate CG4306 – novel

30 Human homologue of Complete gene candidate

4e-25 3242764 (AC005154) similar to protein U28928 (PID:g861306) [Homo

sapiens

35

Putative function No homologies to indicate function

Confirmation by RNAi Only wild type profile observed

158

Example 50 (Category 4)

Line ID

666/19

Category

5

Mitotic defects in brain: anaphase defects

(weak, overcondensation, aneuploidy, lagging chromosomes,

metaphase with bipolar spindle)

Reversion

NR

Map Position

64E1-5

10 Rescue ID I9E

Rescue Sequence CCCTCGTCTACGTCGAAATTCTGGATGCTTCTCGGATTTAGGGTTGTATCTCGA AAACGTTTGACTGCGAATGTCAATATCGATATGCTAACCGATAGCTGTCGATG NTGCTTTTGGCGGTNNTTTTCTTTATATGCTTCTTATGCTTTTACGATTATTATT 15 AGCGCTTATTTGATTGCAAATGCCAAGGAAAGCGTGACTGTGATGGCGAAATG CGGAAAGTACTCCTTAAATCTCATATATCGCATAAAACTATCGGTTCTGGAAT GTTTCGTGTAAGTCTGCGAAGATAGAGATCGATCTATTTTGAGGATACATTTG TTAATATTATAAGGGATTCTTCTACAGGGGTCAGATTGCTTAAAAACACACAG 20 AANAATAAACAAAATATTTCTTTGAAATATTGAAATATTTGAAATANAAAAA CGTATTGACGAGGTAAGCATATTGAAAAAGATAGGAAGGTGATGGAGAAAGT GCACTTATATTGGTCACCAAAGAGCTTATAATCAAAAGATCAATAGATATAAA TATCTTTATATGATATAAAATATAATACATATAATAATATAATATCATATACAATG 25 **GGGCCT**

Genomic hit, Accession No. CSC:AC014815

Associated ORF

- 30 Genscan ORF1 predicted sequences >17:46:43|GENSCAN predicted peptide 1|334 aa MGKDFYKILGLERKASDDEIKKAYRKLALKYHPDKNKSPQAEERFKEIAEAYEVL ${\tt SDKKKRDIFDNYGEDGLKGGQPGPDGGGQPGAYTYQFHGDPRATFAQFFGSSDP}$ FGAFFTGGDNMFSGGOGGNTNEIFWNIGGDDMFAFNAOAPSRKROODPPIEHDLF VSLEEVDKGCIKKMKISRMATGSNGPYKEEKVLRITVKPGWKAGTKITFPQEGDS
- 35 APNKTPADIVFIIRDKPHSLFKREGIDLKYTAQISLKQALCGALVSVPTLQGSRIQV NPNHEIIKPTTTRRINGLGLPVPKEPSRRGDLIVSFDIKFPDTLAPSLQNQLSELLPN

>17:46:43 GENSCAN predicted CDS 1 1 1005 bp

atgggcaaagacttctacaagattctgggcctcgagcgcaaggccagcgatgagatcaagaaggcctaccgcaaactggc 40 actcaaataccatcccgacaagaacaagagcccacaggcggaggagcgcttcaaggagatcgccgaggcgtacgaggtgctg teggacaaaaagaagegegacatettegacaattaeggtgaggatggattgaagggeggacageegggacagatggeggeg g teag cegg age gate cagt tecaegg egate ceg agg gee a catt tige ceagt tett tigg at cegt titting at cegt titting at central control of the cgegttetttaceggeggegataacatgtttagtggeggteagggegataaceaaegagatettetggaacattggeggegaeg atatgtttgcctttaatgcccaggcacccagtcgcaagcgccagcaggatccgcccatcgagcatgatctgttcgtgtggag 45 gaagtggacaagggatgcatcaagaagatgaaaatctcacgcatggccaccggaagcaatgggccgtacaaggaggagaag gtgctgaggatcacagtgaagccgggctggaaggccggtaccaagattaccttcccccaagagggtgattcggcgccaaacaa

159

gacgccagetgacategtetteateattegegacaaacegeattegetgtteaaacgcgagggaategatetaaagtatacagece agateagtetgaagcaggcettgtgeggagcaetggttagtgtgeccaegetgeagggcagcaggatacaggtgaateegaace aegagateateaageceaceacaacgegeeggateaacggaetgggtetgeeggtgeccaaggagccategaggegggggatetgategteteettegacattaagttteeegacacatggaaceagtetgeagaateagetgteegagetgetgeeaactag

Drosophila Gene Hit rescue sequence: fasciclin I (FasI) (M32311) TBLASTN with

ORF1: DnaJ homolog (DROJ1) (U34904)

Human Homologue TBLASTN with ORF1: DnaJ-like heat shock protein 40 (HLJ1)

(U40992.2)

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Annotated *Drosophila* genome genomic segment AE003565

Annotated Drosophila genome Complete gene candidate CG10578 - DnaJ-1 a

chaperone putatively involved in protein folding. Stimulates

activity of HSP70

Human homologue of Complete gene candidate

8e-94 1706473 P25685 DNJ1_HUMAN DNAJ PROTEIN HOMOLOG 1 (HDJ-1) (HEAT SHOCK PROTEIN 40) (HSP40)

20

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Putative function Chaperone involved in protein folding

Confirmation by RNAi

Almost no G1 peak, increased G2/M indicating G2/M arrest

160

Example 51 (Category 4)

Line ID 714/11

Category Meiotic defects in testis: cytokinesis defects, abnormal spindles

(Ab-01/04)

Reversion

Map Position 66A10-15

Rescue ID 2A4E

10 Rescue Sequence

5

AACCAGAACGAAACTCCAATGCAGTTTCATTTTGTCAGTTTAATCATTAAACA
AAGAACTGCGCAACCGATCGCAACTAGCTCGTGGACTCTTGTTCTCCCAATAA
TTGGTATGTTTTCCATTTTGCGTTAACATGGAAAATGTGTGAAAAAGCTTTTTCC
CCCTCCAAAAGAAGCGTACTGAACTAAGCTTTCGGTGGTTAGTAATAGTAGTC

15 GTTATATCTTATTTTCTTATTTACGTGCAGCTGCAATCATTGGCTGCGTCACTT
TGGCGTCAGCTATAAACTGGTGGATCAACTCGGCGGCCTCCAAAAGCTGCGCA
TCTGCTCCAGACACTTTAGCCAACGCCAGGAGATGGCCAAAACCCGCATCAA
GATGACGCCGCTGCGCAAGTCCTCGTCCTCCAAGGGCATTGTGCTACCCATTA
ATGCCGCTGGAGGGTCGTCATTGCAGGCGCCTTAGCACGAGGAGGGGGCC
20 A

Genomic hit, Accession No. AC012390

Associated ORF

- 25 Genscan ORF1 predicted sequences >19:47:45|GENSCAN_predicted_peptide_2|711_aa MRSHQAVGNLLLAADEALPAVQSASVYVVWMAEQPLSPGQSYDIKIADSPSVSS KSITDNGADVQWFAFEHSQYYQGVQQMFLSALERIDSEFLITLIKRCPYHVDSLVQ LSEVCKMTEDFSLASELLERALLLLESSLHINFSLTSGNCRLDYRRQENRSFYIVLF KHAQYLEERACSRTAFEISKLLLSLQPDTDPLAMILPNQPDQCTGNMTQLQQAGK
- 30 IRKRSEKQFPIGTEPRGTDALRFTLQTLASAGRDITWNIKRLQGSRVTGAAQGYLI DKKTAVQYKITIIAHLKDPNIDQLFDSSGDGKADLHGSTPDWGCQAMMADAISR YKEGNPVFYYTWTPYWVSNELKPGKDVVWLQVPFSALPGDKNADTKLPNAGGI EGLIADEEVQVLDALCDAPCVGVSHSCRLLDGNRRGNNELRLFIPGKSQFGVADG CADKQSVMEYHAAKTGHTKFSESEEEKKALTEEEKKAQLALIEEKLKQKRIEREE
- 35 REKIEALQREKNRIKSGKDMTEAKRRMEELEMKKIVEQRKREKDEEKAARDRVK AQIEADKAARKAREQKELGNAEPAPSVSSTTVSSPPAGVKSPPRDYTETRIQGASA ILAAAAPYYQPPAVPQDVQPDRPIGYGAFGVVCGSHISGWHCSAGHYEDGNENFE CLKTFSTSDRIGCEWRWAAATVLAATCISPNGRCGHYKRVRRRIKTNITTT
- 40 >19:47:45|GENSCAN predicted CDS 2|2136 bp
 - atgagategeateaageegttggeaatetgetggeggeagaegaagegttaceggeggtgeagagegegteggtgtatgtg gtatggatggeggaacageegettteteeagggeagagttacgacateaaaattgeegacteteeateggtgteeteeaagtetate acagataatggageggaegtteaatggtttgeetttgageatagecaataetaceagggagtgeageaaatgtteetttetgeteteg agegeattgaeteggaatttetgateacaettateaaaegetgeeeetateatgtegaeteettggtteaaeteagegaagtatgeaa
- 45 gatgacegaagactttteettggeeteegaactgettgagegegeeetteteettetggaategtegetgeacateaactteagtttga egtegggeaactgeegaetggactaceggagaacaggaaaacegateettetacategtgetgtteaageaegegeagtacetgg

aggaacgagcttgcagccgcaccgccttcgagatctccaaactgctcctgagtcttcagccagacacagatcctcttgccatgatt ctaccaaatcagccggatcaatgtaccggcaatatgacgcagctgcagcaggcgggcaaaatccgtaagcgctcagaaaagca gtttccgatcggtactgaaccgcggtactgacgcgtttcaccctgcagacactggcgtctgccggtactgacactcacct5 aatcaccatcatcgctcatctgaaagatccgaatatcgaccaactgttcgattcaagcggcgacggaaaagcggatttacacggta gtaccccagactggggctgccaagctatgatggccgacgccatcagtcgctacaaagagggcaacccggtgttttattacacctg gacgccgtactgggtgagtaacgaactgaagccgggcaaagatgtcgtctggttgcaggtgccgttctccgcactgccgggcga ttgtgatgcgccgtgtgttggtgtctcccactcgtgccgactccttgatgccaatcgccgagggaataatgaactgcggctctttatt 10 accaaattctccgaatcggaggagaaaagaaggcgctcaccgaggaggagaagaaggcccagctggccctcatcgaggag aagetcaageagaaacgeategaacgegaggagegegagaaaategaageeetgeagegggaaaagaategeateaagtee ggcaaggacatgaccgaggccaagcggcgatggaggagttggagatgaagaagatcgttgagcagcgcaagcgcgaaaa ggacgaggagaaggcggcccgcgatcgggtaaaggctcaaattgaggcggacaaggcagcacgcaaggctagagaacaaa 15 gagactacaccgaaacccgcatccagggcgccagcgcaatcttggccgcagcggctccctactatcaaccgccggctgttccc caggatgttcagccggatcgtcctatcggctatggagcattcggagttgtctgcggttcccacatcagcggctggcattgttctgcg gggcattatgaagatggtaatgaaaatttcgagtgcctcaagacattttcgacttctgaccgcattggctgcgaatggagatgggcg gcagcaactgttcttgccgcaacctgcattagcccgaacggccgttgcgggcattataaacgcgtacgtcgtcgcattaaaacaaa 20 cataacaactacgtga

Drosophila Gene Hit rescue sequence and BLASTX with EST: BIP1 (Y14998),

BLASTX with genomic sequence matches BIP.

Human Homologue BLASTX with BIP1: alanine:glyoxylate aminotransferase

(X53414)?

Drosophila EST GM04749 (AA695904), GM13608 (AA803601)

Annotated *Drosophila* genome genomic segment AE003556 Annotated *Drosophila* genome Complete gene candidate CG7574 - bip1 unknown function

CG13681 - unknown

Human homologue of Complete gene candidate none

35 Putative function

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no homologies to indicate functions, Drosophila Bip1 interacts with transcriptional activator Bric-a-brac which is required for ovariole formation

40 **Confirmation by RNAi** Both show reduction in G1 and G2/M iondicating fewer cycling cells

162

PCT/GB01/01297

Example 52 (Category 4)

Line ID 763/4

Category Meiotic defects in testis: segregation defects

(overcondensation, fewer anaphases)

Reversion R **Map Position** 90F

Rescue ID 2F5E-1

10 Rescue Sequence

WO 01/72774

Genomic hit, Accession No. AC006495

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Associated ORF

Genscan ORF1 predicted sequences >22:47:02|GENSCAN_predicted_peptide_3|283_aa MTERENNVYKAKLAEQAERYDEMVEAMKKVASMDVELTVEERNLLSVAYKNVI GARRASWRIITSIEQKEENKGAEEKLEMIKTYRGQVEKELRDICSDILNVLEKHLIP CATSGESKVFYYKMKGDYHRYLAEFATGSDRKDAAENSLIAYKAASDIAMNDLP PTHPIRLGLALNFSVFYYEILNSPDRACRLAKAAFDDAIAELDTLSEESYKDSTLIM QLLRDNLTLWTSDMQAEEIPIPKLPDRQSKTTLIFSPRSQVNPKILHKNNTIIGRVIC SVFA

- >22:47:02|GENSCAN_predicted_CDS_3|852_bp
 atgactgageggggaaacaatgtgtacaaggcaaagetggcegaacaggccgagegctacgacgaaatggtggaggccatga
 agaaggtegcetecatggacgtagagetgacegtegaggagegaaatetgetgteggtggegtacaagaatgtgattggagcac
 geegtgeetegtggegeateateacetegategaacagaaggaggagaaacaagggggecgaggagaaattggagatgateaa
 aacetacegeggacaggtggagaaggagetgegegacatetgeteggatatactgaacgtgetegagaagcateteattecatg
 cgccacatceggegaaagcaaagtattetactataagatgaagggegactaccategetacetggecgaattegecaceggetee
 gaccgcaaggatgeggeaggaaactegetgattgeetacaaggeggecagegatattgecatgaacgatetgecaceaacaca
 ceccatecgtttgggettggeattgaactteteggtgttetactatgagattetaactegecggaccgcgettgecgettggegaaa
 gecgetttegatgatgecattgecgattggatacactgagegaagagagtacaaagactegacacteatcatgagetgetge
 gegacaaceteacattatggacgtecgatatgcaggcagaagagagattecgattecaaaacteceegacagacagtecaaaacca
- 40 cattgatttttagccccgaagtcaagtaaacccaaagattctccacaagaacaacaccatcatcggcagagttatctgtagcgtgtt tgcgtga

Drosophila Gene Hit rescue sequence: 14-3-3 epsilon isoform gene (U84898)

TBLASTN with ORF1: 14-3-3.

45 **Human Homologue** TBLASTN with ORF1 and BLASTX with 14-3-3: epsilon isoform 14-3-3 protein (U43430.1)

163

	Annotated <i>Drosophila</i> genome genomic segment Annotated <i>Drosophila</i> genome Complete gene candida	AE003721 ate CG8045 complex gene appears to encode 3 things:
5		Transcript: CT24102 unknown Transcript CT24072:
		transcription factor RNA polymerase II transcription factor,
10		Transcript: CT24092: diacylglycerol-
		activated/phosholipid dependent protein kinase C inhibitor /14-3-3 protein
15		epsilon (suppresspr of ras)
	Human homologue of Complete gene candidate	CT24092: e-119 NP_006752.1 tyrosine 3- monooxygenase/tryptophan 5-
20		monooxygenase activation protein, epsilon polypeptide; 14-3-3 epsilon [Homo sapiens
25	Putative function transcription factor, or 14-3-3 proteins which associate with cdc25 phosphatases	
	Confirmation by RNAi CT24102: wild type profile only, CT24072: Loss of G1 peak CT24092: Increase of G1 peak	

164

Example 53 (Category 4)

Line ID 951/8

Category Mitotic defects in brain:

(some overcondensation, anaphase bridge, metaphase with

swollen chromosome and bipolar spindle)

Reversion NR **Map Position** 73D

10 Rescue ID 2E8S

Rescue Sequence

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25 Genomic hit, Accession No. CSC:AC015272

Associated ORF

Genscan ORF1 predicted sequences

- >23:03:05|GENSCAN_predicted_peptide_1|602_aaMGFDMATRFMDILKLTFKPFKTN
 YTEEKYFNDKLRSSKNIERRYILDVGFRGPTAVTYNPIWVISFKYEQRKLSTAIYSV
 IKTKSGPVRGVKRNTIWGGSYFSFEKIPFAKPPVGDLRFKAPEAVEPWDQELDCTS
 PADKPLQTHMFFRKYAGSEDCLYLNVYVKDLQPDKLRPVMVWIYGGGYQVGEA
 SRGLDVVIVTVAYRLGALGFLSLDDPQLNVPGNAGLKDQIMALRWVQQNIEAFG
 GDSNNITLFGESAGGASTHFLALSPQTEGLIHKAIVMSGSVLCPWTQPPRNNWAY
- 35 RLAQKLGYTGDNKDKAIFEFLRSMSGGEIVKATATVLSNDEKHHRILFAFGPVVE PYTTEHTVVAKQPHELMQNSWSHRIPMMFGGTSFEGLLFYPEVSRRPATLDEVGN CKNLLPSDLGLNLDPKLRENYGLQLKKAYFGDEPCNQANMMKFLELCSYREFW HPIYRAALNRVRQSSAPTYLYRFDHDSKLCNAIRIVLCGHQMRGVCHGDDLCYIF HSMLSHQSAPDSPEHKVITGMVDVWTSFAAHGDPNCESIKSLKFAPIENVTNFKC
- 40 LNIGDQFEVMALPELQKIEPVWNSFYAPNKL

>23:03:05|GENSCAN predicted CDS 1|1809 bp

atgggattcgatatggcaacacgctttatggatatactaaagctgacctttaagccatttaaaacgaactacactgaagaaaagtattt caatgacaaactcagatcttcgaaaaatattgaaaggcgttatatcttggatgttggctttcgcggacccacagcagtcacgtacaat ccaatctgggtaataagcttcaagtacgagcagcgcaaattgtcaacagcaatatattccgtcataaagacgaaatcaggtcctgtg cggggagtgaagagaaacacaatctggggaggaagctacttcagtttcgagaagatacccttcgcaaagcctccggtgggagat

165

ctgcgcttcaaggccccggaagcagtggagccatgggatcaggaattggattgcacttcgccggcagacaagccccttcagaca cacatgttttt cagaaaatac gcgggctcagaggactgcctctacttaaatgtgtatgtcaaagatctgcagccggataaactgcgtcccgtgatggtttggatctacggaggaggctatcaagttggcgaagcttctcgaggattggatgtggtcatagtcaccgttgcttatcg actgggtgccttgggcttcctcagcctggatgatccccaactaaacgttcccggaaatgcaggtctcaaggatcaaatcatggccc tgcgatgggtgcaacaaaacatcgaagcattcggcggtgattccaacaatattacactctttggcgaaagtgccggcggagcctc gacccacttccttgcactaagtccccaaactgaaggtcttatccacaaagctatcgttatgtcgggcagtgttttgtgcccctggacg caaccaccgagaaataattgggcttataggctggcccaaaaattgggatacaccggtgacaataaggacaaggcgatctttgagt ttetgegateaatgagtggeggggagattgteaaggeeacegeaacagtteteageaacgatgaaaageateateggateettttegcettcggacctgtcgtagaaccatatactaccgagcacactgtggtcgctaaaccaccgcatgaactgatgcagaatagctggagtcacaggatacccatgatgtttggaggcacgagcttcgagggattgctattctatccagaggtttcaaggcggccagcaaccctc gatgaggtgggtaactgcaagaatctgctaccgagcgatctcggtcttaacctagatcccaaactgcgtgagaactacggcttgca actgaagaaggegtattteggegaegaaceetgtaaceaggeaaacatgatgaagtttetegagetatgeteatategagagttetg geaccetatatacagggcagetttgaaccgtgtccggcaatccagcgcacccacgtatctgtatcgattcgatcacgattccaaact gtgcaacgccattaggattgtactttgcggccatcagatgcgaggtgtttgtcatggtgacgatctgtgctatattttccacagcatgtt gtcgcatcaatccgctcccgattctccggaacacaaggttataaccggaatggtcgacgtttggacgagtttcgcagcccacgga gatcccaactgcgaaagtataaaatcactcaagtttgcacccatcgaaaacgtaaccaactttaagtgtctcaatattggggatcagt

Drosophila Gene Hit TBLASTN with ORF1: alpha esterase (aE10) gene (U51054)
 Human Homologue TBLASTN with ORF1 and BLASTX with U51054: bile salt-dependent lipase (S79774)

Annotated *Drosophila* genome genomic segment AE003671

Annotated *Drosophila* genome Complete gene candidate CG1131 - alpha esterase 10

Human homologue of Complete gene candidate

4e-48 4557239 ref|NP_000656.1|pACHE| acetylcholinesterase (YT blood group) precursor >gi|113037|s

Putative function

alpha esterase

Confirmation by RNAi

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Only wild type profiles observed

166

CATEGORY 5: SMALL IMAGINAL DISCS (BLOCK TO PROLIFERATION)

Example 54 (Category 5)

Line ID 113/20

Category 2nd chromosome, small imaginal discs

Reversion R **Map Position** 50D/E

Rescue ID EcoR1

10 Rescue Sequence 1

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CTGAGGCNCTTTGCCAATATGTGTATATTGGGCGGGGNACATGCGTNAATCGG
TTAAAGCCGCTACTTACATTCTGTTCTTTGCATCTCCCCCATCCACAGCTATAA
AGCAAGATGAGCTACGCCGCTGATGTGCTGAACTCGGCCCATTTGGGAGCTCC
ATGGTGGTGGCGACGCCGAGTTGCGTCGTCCATTCGATCCCACGGNCCATGAT
TTGGATGCATCCTTCCGCCTTACACGCTTCGCANATCTAAAGGGGCGCGGCTG
CAAAGTGCCCGCAAGGAAGTNGCTCCCCCACCT

Rescue ID BamH1

Rescue Sequence 2

20 CCACCTGTACCACAGCGCTCANACGTGTATGTACACGGATTTTCTGCCGCGT GTGTGTAGCGCGGCCCGTGATTGGCTGCAGTCGCGATGGCGGCTAAAACGGG CGAAGTCAGTATTTCTCCCTGTCGACGANGCGAGCAACGTGAACAATGCCCAC TCATTTCAATTGCAAAAATGCCAAAAAGTGCGCGCTTTGAATTGGCCATTTGGT TCGTTGCGTTCGTTTGTCTTTTGGTACTTACGTTTGCTTGTGCGATTGTACAAA

25 GATAATTGTAGAGTAACGTTAGCAAATTATATTTATTTTGCGCCTGGTTTTTGC
TTTTCCAACGANCGAGATGTCACAACAGGGTTGTATTANCGTGTGCGGCTGAT
TCGATATTTGGGATGCCGATTGTCTGAAGCGANGGTTCAACGGGGCTGCCAAC
TCCCCCGAAAATCTATCNATGGTATTGTGCGCCAAGGGTAAAATAAATAAAAA
TATGTTAAAACCGCGGAATAAATGGGGGAACCGAAGTGGAAACTGTGGTTCA

30 CAGTGCTCTGACTTTCGGGAGCAGTTAATATAGTTGGCATTAATTCAATTAGA GCTCCAAAGTGCTGGTCACAAAGAACGCACAAGAACGGGCCATGAAAAACCT GTTGCGCCAGCAGAACGAAAAGTAAAAATTAGAAGAAACCAT

Genomic hit, Accession No. CSC:AC017131

35 Drosophila Gene Hit rescue sequence: selenophosphate synthetase (ptuf1) (U91994)

Human Homologue BLASTX with U91994: SELENIDE, WATER DIKINASE 1

(SELENOPHOSPHATE SYNTHETASE 1) (SELENIUM

DONOR PROTEIN 1) (P49903)

Drosophila EST LD46437 (AI514756 similar by BLASTN to U91994

selenophosphate synthetase (ptuf1) gene)

Annotated Drosophila genome genomic segment

AE003815

167

Annotated *Drosophila* genome Complete gene candidate CG8553 selD selenophosphate synthetase

Human homologue of Complete gene candidate

1711372 P49903 SELD_HUMAN

SELENIDE, WATER

DIKINASE

(SELENOPHOSPHATE SYNTHETASE (1e-159)

10

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Putative function

selenophosphate synthetase

Confirmation by RNAi

Only wild type profiles were observed

168

Example 55 (Category 5)

Line ID 121/1

5 Category 2nd chromosome, small imaginal discs

Reversion NR **Map Position** 60B

Rescue ID BamH1

10 Rescue Sequence

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TCCTGTGCACTCATATTGATTTGCCTTGTCAAGTGGCTAAAGAAATATTAAATG TTTGTTATTCTGTTGCTAGCGCTCCGACAGTCTGGCAGCACTGCTCGCTGTCG ATAGTTCAACTGAGTTGCTGTTTCATCGAACAGAGCTGCCAACTCTATTTTGT AGCTGGCCAGCCAGGATTGCCAGAGTAAGGCCCTCAAATCAGCTGTTTTGTGT TTTGATTTTATTTTGAAAGTCCTAGTTTAAAATTATGCTTTCTCCGACAGATCA GCACAAATAATACTAATAAAGCTCACAATGCTAAGGTTGTGCCTTCCAACTCG AGCTGGATATGTGCGTAAGTAAGGACTTTACGTCTATAAAACTTGTTATGTAA AGTAAATGTTTGCCTATTGCGCAATTTCTCCAACGAAAAACCCAGAAAACCNA AACCCCCTTNAAANTTTGGAATATNCCCAATGAATGCAGCACCCGTGAAATCC GTAATGCCTTTGTCCAGCTCTCCAAATTGGTAAGTAACTCCAAGATCCAAAGG

20 AGCCTCCTAAACCCTGCCCTTTCCACAGTACCACCCAGATGTTAAGAGCAATG CTGCGTGTCCGGAGCGCACAGCCCGATTTGTTCAGATCTCCGAGGCGTACAAG AACCTGATAAAGCCGGAACGGAAGGAAAAA

25 Genomic hit, Accession No. CSC:AC020499

Drosophila Gene Hit rescue sequence: DnaJ60 gene for dnaJ-like protein (Y11900)

Annotated *Drosophila* genome genomic segment AE003463 Annotated Drosophila genome Complete gene candidate CG12240 - DnaJ60

30 CG13570 - spaghetti ser/thr

phosphatase

Human homologue of Complete gene candidate CG12240-4827026

ref[NP 005138.1|pTID1| tumorous imaginal discs (Drosophila) homolog >gi|3372677 (AF061749) 7e-

80

40 CG1116-2495728

HYPOTHETICAL PROTEIN

KIAA0258(aa)

45 **Putative function** CG12240: Chaperone involved in protein folding

CG13570: serine/threonine phosphatase

169

CG12240: Marked reduction in G1 and G2/M peaks indicating fewer cycling cells CG13570: Marked increase in G1 peak Confirmation by RNAi

5

170

Example 56 (Category 5)

Line ID 127/2

Category 2nd chromosome, small imaginal discs

5 **Reversion** NR **Map Position** 57F

Rescue ID EcoR1

Rescue Sequence 1

10 GCCGGTGGGCCCACACTTGTNCGCCCGCGCATCGGCTGTCTGTGGGAGTGCGA NCGAGTCAGATAGTAGATCCGATGCGCTCTCCAGATACTTTTTGAACACTGAA ATCCTAGTCGCCTCACGCGAAGAGAACTATGTCATGATCAGATATCGGTGTAT GCATTCTATATTATGTACTTCGAAATATGTAATTTATTAAGTTTTCGCTATACT 15 TTTCATTCAAATTGGCAAAAACCAATTCAAAGGTTTTCAATATTTTCGAAAAG AACTTTATTATCGGATAAACAAATGTAAGCCAAATNACAACGTTNTATGATAC TCCCAAAGATCCGCNCTNTTAAAGTGGCCTAAAAATAGCTGACGCATTAANCC ATAGGCGCTTCGCTTCTCAAGATAAAACCTGGCGTGCTCAACTCAAGAAACAA 20 ATATGTGGTTATATACATATACATATATGGGGCATATAACCGATGTGTGAC GTGACATTGGCTCGTTCTATTCACATACTTAAACACTAAATGCAAACCTATCA AAAACCNACTACACTAAGCGAAAAACGGCAGANATAGTTAAGGAAAGTGGTC CA

25 Rescue ID BamH1

Rescue Sequence 2

CTTCTTTTCTCAAAAAACGTCGCTCGNGTCCCNCAATCGTTTTACAAACTTCGC TCGGAACGGACGTGTGCGCGCTCTGAAAGGAAAAAGTGAAAAAGTGTGTGAC AAAGTGCAAATAAGCCACAACGCGCATGTGAGAAATCAAATTTAATTGAGAA 30 GCATCAAAAATTGTATACATATCGAGCGTATCCACATCGCTGTATGTGTGAGT GTGCCAGTGCTAGTGTGTTTTCCCTTTTCGCCGTGGAAAATATGAAAACTGA ATGAAAAACTGAATCGCAGTCAGCCAGAGCCGAATTGGAAAAGAGTAACTCG CATTGGGGACACGAAGAGGTGTCTCGAAAAAGGTAAAATCTTTTACACAGAA ACGACGCCAGAAAGCGATTAGCGATTTNTGACTATGTGTGAGTGTAATTTC 35 GGTCTACGGCTGTGTGTCTCCATTTTATTTAACNTTTTGTTTCCCNGTTNGNTC CACNGTAAAAATAGCTAAAAAAAAAGGGCAAGTACTCTTGGCGCGCTCTCCC TCTCTCTTTGTTGGTCGTGACTGCGACGTCACCGTTCACGTAGAATCGTTTTCA AGTGGCGTTTCTTTCTTTTTAATGTGCTGCTTCTTGCTTCTTCTTC TTGCCTTTGGCTATCTGCTTTGTTTTGAAATACGTCCATGTTATTCCAGTGTCTG 40 TGCCAAATGTGTGCGANATGATCTCTACTT

Genomic hit, Accession No. AC009732

Associated ORF

45 Genscan ORF1 predicted sequence >/tmp/aaaaafrla|GENSCAN predicted peptide 2|456 aa

MOTKGPITDADCIRGMACRALAGLARSDRVROIVSKLPLFASGOLOTLMRDPILO EKRAEHVIFOKYALELLERVSGKTKPLNNPLDPSLSNMHKANVIAQTRIQYNKQQ LYQLIFEHLESNGLSQTAQMLQREVGLPLQTPTTRSFHQSPFDYKSLPSGSSSLSRN RLRSRMQDVNAAIMGNGDLNRSFGEDSSPAGAGGSNAGDGVSIPNFSSLNTTQTP IKIRRTDRSSVSRSIOKOAMEPGGMSVGLAEDGOLHPKRITLNTIVTEYLTNQHSL CNNPVTTCPOFDLYEPHKCPDPKPSRLLSSNYNLTSRHARTQAGFNTSRFDRRYV HTHFSPWRSIRSADYEDLEFTCCDLAGKYIIVGTQQGDGRVFNMNDGVEQFFSNC HNFSVDAIKANRAGDLVITSSFWRTPTSILWSIADDEFKLKLRLPDVTYCEFSQTV **QDRLLGTQNEVY**

10

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>/tmp/aaaaafrla|GENSCAN predicted CDS 2|1371 bp gggtcaggcagatcgtcagcaagcttccactctttgccagcggacaactccagacgctgatgcgggatcccatactccaggaga aggggggaacatgtaatcttccaaaagtacgcattggagttgctagaacgagtgtcgggtaagacgaaaccgctaaataatcctttggatccatcgctgtccaacatgcacaaggccaatgtaatcgcccagacacgcatccagtataacaagcagcagctgtatcagcttatcttcgagcacctggaaagcaacggtctctcccagacagcccaaatgctgcaacgggaggtgggtcttccgctacagactcc cactacgcgcagttttcatcaatcacctttcgactacaaaagtcttcccagtggtagtagctcgctgtctagaaatcgtctgcgaagc cgcatgcaagatgtgaacgcagcgataatggggcaatggagacttaaacagaagttttggtgaggactcctcgccggcaggagcc ggtggtagcaatgcgggagatggagtcagcataccaaattttagctcccttaacacacagcagacgcccataaaaataaggagg acggatagaagttcagttagccgctctatccagaagcaggcaatggagcctggtggcatgtcagttggtcttgccgaagatggtca acetgcccgcagtttgatttgtacgagccgcacaagtgtccagatccgaagcccagccgattgctaagctcgaactacaacctga ctag tegg cat get cga accea ag cegg att taat accag teget tt ga ceg teget at gt gea cae gea cttt teaccat ge get accea ge get at get gea cae gea ctt general ggeattegateggeggaetaegaggaeetagagtteaeetgttgegatttggegggtaaataeateattgtgggeaegeageaggg cgacggacgagtgttcaacatgaacgatggcgtggagcagttcttctccaactgtcacaactttagcgttgatgctattaaggctaatagage cgg agacttgg teat cacate tagette tgg egea cace caceag cattet at gg te tattge gg acgat gag tte aagetaaagttgcgacttcccgatgtcacgtactgtgagttcagtcaaacggtgcaggatcgtttgttgggcacccagaatgaggtatactaa

corresponds to CG10082

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Drosophila EST several including SD04293 (AI532704)

Annotated Drosophila genome genomic segment

AE003454

Annotated Drosophila genome Complete gene candidate CG10082 - novel protein with

homology to enhancer Pi

uptake

Human homologue of Complete gene candidate

1665793 dbj|BAA13393| (D87452) Similar to S.cerevisiae YD9335.03c

40

protein (S54640) [Homo

sapiens] (2e-43)

45 **Putative function** Putative phosphatase or enhancer of Pi uptake protein

Reduced G1 and G2/M peaks indicating fewer cycling cells Confirmation by RNAi

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Example 57 (Category 5)

Line ID 131/8

Category 2nd chromosome, small imaginal discs

5 Reversion R Map Position 60A

Rescue ID BamH1

Rescue Sequence 1

10 CACGATTGCNGGCCCATCGAAGTGTGGGTCTATCGATACTCGTGGGTAAATAA ACAAGTTCTGAACTGCGATTTCGGGGGTTTGAGGGGTCAATTGTCCCCTGTGT TGGAATGTGTTCCTAAATCTACACAAACACTCCCTAAGCTTATCCTAAACTTAT AAATATTGGTTGCTATTTAAACCCCATTTCACGGTTATCCAGCACGCCCCTGA ACTGTGACCCACATCCCCGATTTTAGTGACTAGTTTTATACTTATCGTGGTTGG CATTTGGTACACTACACTTTCTTATTCACCTAGATCGCCGACTCCGCGCACGGT 15 CGCGCTCCCGTTCCCGATCTCGGCTGCGACTGCGGTCGCGATCCCGTT CCCGGTCGCGGCGCCCCCANATCCGGATCCCTAANCGGCANCNGT CNTGGTGGCAATCNNGGAATGTTCCGGGGNNCCNCTACCNCAGTGNAATCAC TGGTACGTCCCACCGCNAAACTCCGCCCANTGCGGTTGCCGGAACGGGTGGC 20 ANTGCCAATGGGTCGCTGCAGAAGGTACCATCACAGCAATCGCTCACGGANC CCGAAGACTGCCTCTGCCGCCGGCTGGGCCACTCATACACGCTACACGGTCG GAAATACTACATTGATCACAATGCGCATACCACGCACTGGAATCATCCGTTGG

25 Rescue ID EcoR1

Rescue Sequence 2

GAACGC

AATTGATTTCCGGACATATAAACAGAATCCAGAACTCATCCGGCAGCAGGCTC
AGTCAGGCCAGTAAATCCGAAAAGAGAGTAACCAGCAGGAAAAAGAGAATCC
ACGTAAATACAGAGAAAATGGCTCTACGCGTCCAATTCGAGAACAACGACGA
30 CATCGGCGTATTCACTAAACTAACCAACACATACTGCCTGGTGGCCATCGGTG
GATCCGAGACCTTCTACAGCGCCTTCGAGGCGGAGCTGGGCGACACCATCCCG
GTGGTGCATGCGAATGTGGGCGGCTGCCGGATCATCGGCCGCCTCACCGTGGG
CAACCGCAACGGCCTGCTGGTGCCCAACTCCACCACCGACGAAGAGCTGCAA
CACCTGCGTTACANCCTGCCANAACCCCGGAAANATTTATCGTGTGGAAGAAC
35 GCCTGTCCGCGCTGGGCAACGTTATCGCCTGCAATGATTATGTGGCCCTGGTG
CACCCGGATCTGGACAAGGAGACCGAGGAGATCATCGCGGACGTGCTCAAAG
TANANGTCTTCCGCCAGACCATTGCCGACAACTCACTGGTGGGCTCTTACGCC
GTGCTGAGCAACCAGGGGGGCATGGTGCATCCCAAGACNAGCATTCAGGAAC
AGGACAACTGTCGTCCCTGCTGCAGGTTCC

40

Genomic hit, Accession No. CSC:AC020517

Associated ORF

Genscan ORF1 predicted sequences >22:13:05|GENSCAN_predicted_peptide_4|357_aa
45 MALRVQFENNDDIGVFTKLTNTYCLVAIGGSETFYSAFEAELGDTIPVVHANVGG
CRIIGRLTVGNRNGLLVPNSTTDEELQHLRNSLPDAVKIYRVEERLSALGNVIACN

173

DYVALVHPDLDKETEEIIADVLKVEVFROTIADNSLVGSYAVLSNOGGMVHPKTS IQDQDELSSLLQVPLVAGTVNRGSEVLAAGMVVNDWLSFVGMNTTATEISVIESV FKLNQAQPATVTTKLRAALIEDISRSRVAGGGGGGGGGGGGSSGGNSSSGPSTSRRTT RNNAAATAADRPKINEADLEGKSPEEVEMLKTMGFCTFDTTKNRKVEGNDVGEV HVILKRKYRQYMNRKGGFNRPLDFVA

>22:13:05|GENSCAN predicted CDS 4|1074 bp

atggctctacgcgtccaattcgagaacaacgacgacatcggcgtcttcactaaactaaccaacacatactgcctggtggccatcgg tggatccgagaccttctacagcgccttcgaggcggagctgggcgacaccatcccggtggtgcatgcgaatgtgggcggctgcc10 ggat categge cgecteac egt gggeaac egcaa eggect get ggt geceaac te cacea cegae gag gag et geaac acctgegtaacagectgecagacgecgtgaagatttategtgtggaggagegectgteegegetgggeaacgttategeetgeaatgat tatgtggccctggtgcacccggatctggacaaggagaccgaggagatcatcgcggacgtgctcaaagtagaggtcttccgccag accattgccgacaactcactggtgggctcttacgccgtgctgagcaaccagggcggcatggtgcatcccaagacgagcattcag gaccaggacgaactgtcgtcctgctgcaggttccctcgtggccggaacagtgaaccggggcagcgaagtactcgccgccg 15 geatggtegteaacgaetggeteteettegtgggeatgaacaecaeggeeacagagateteegtgategagagegtetteaagett aggaacaatgcggcggccacagctgccgaccggcccaagatcaacgaggcggacctggagggtaaatcgccggaagaggt cgagatgctgaagacaatgggattctgcacgttcgacaccaccaagaacaggaaggtcgagggcaacgatgtcggagaagtgc 20 atgtaateetcaagegaaagtacegecagtacatgaategeaagggtggetteaaceggeegetegatttegtggeatag

Drosophila Gene Hit rescue sequence and TBLASTN with ORF1: b(2)gcn

(EUKARYOTIC TRANSLATION INITIATION FACTOR 6

)((X97641)

25 BLASTX with X97641: integrin beta 4 binding protein (HUMAN Human Homologue

EUKARYOTIC TRANSLATION INITIATION FACTOR 6)

(NP 002203.1)

Drosophila EST GH08760 (AI109537 similar by BLASTN to X97641

"D.melanogaster b(2)gcn gene.")

30

40

5

Annotated Drosophila genome genomic segment AE003462

Annotated Drosophila genome Complete gene candidate CG17611 - bcgn benign

gonadal neoplasia homology

to Eif6 translation factor

35 Human homologue of Complete gene candidate

6016331 EUKARYOTIC

TRANSLATION

INITIATION FACTOR 6 (EIF-6)(aa) and 4504771 |ref|NP 002203.1|pITGB4BP|

integrin beta 4 binding

protein(aa)

45 Putative function eukaryotic translation initiation factor 6 (eif-6)(aa)

Confirmation by RNAi Slightly reduced G1 and increased G2/M indicating block in G2/M

174

Example 58 (Category 5)

Line ID

135/25

Category

2nd chromosome, small imaginal discs

Reversion

NR 24A

Map Position

Rescue ID

EcoR1

Rescue Sequence

10 ATAACATGGGCNCTGGTTTTTAAGTNAAGCTCTANTNATTGGCCCCCATTCTTA NNCTCTCTCGCTCTTCTCGCTCTTTCGCCTGCTCTCTCGCCTGATTATTCTGC TTGGTCGGCTGATGGTTTTTNGTTTTNATCTGGTGTATTTTCTGCGTAGTTTATG ACAAACCGGCTGGTTCTTGTTGTTATTGCCGTATTCTAATATATTCCCCTATTG TTCTTATTTTGTTGCAGCCTGCACACCTCGGAGGTTCTAGATGATAAGGGGTG TAGCGATGGTGGGGGGCTGTCTTGANGGGCTTCTCGCCTTGAGCTCTTGTTTAT 15 CTTTGGTCATTTGTTATTGTTTAATGCACGGCAATATTATTGGTAAACAAGTTA GCCAACAGCACTAAACGCCAATCGCATTCTTTTCTAAAAACCAAGTCTATTGT CGATCTTGCTAGGGAAATGATGATGACTCAGGTGCAATTGGGATCTTATCTAT GGCTGTCTGGGAATCAAGAAGTGTTCCCGCAGAATTCGTGAANTACTGCCGCT 20 CTCTCCATGGGGCCATTATTTGCACTCGTTTTNCGCGAAATACCATNAATTAGC ATAAAGACACGTCGCCGGCAATCGTGACGTAGGCTATNAATGCCTTCTATGCA TGTGCNAACTCGCGGAAGCATAGCAATTTGAAGGAACAATATTTCANTGCAG **GTTTTAATGGGCTAAAAA**

25 Genomic hit, Accession No. CSC:AC014199

Associated ORF

Genscan ORF1 predicted sequences >20:54:54|GENSCAN predicted peptide 3|117 aa MSASPTAROAITOVMPMITRKVVISDPIQMPEVYSSTPGGTLYSTTPGGTKLIYER AFMKNLRGSPLSQTPPSNVPSCLLRGTPRTPFRKCVPVPTELIKQTKSLKIEDQEQF QLDL

>20:54:54|GENSCAN predicted CDS 3|354 bp

atgtccgcttcacccaccgcccgtcaagccatcacccaggttatgcccatgatcaccaggaaggttgtcatctcggatccgatcca gatgcccgaggtgtactcctcgacgccgggggaacctctactccaccactcctggaggcaccaaacttactacgaggggc 35 tt cat gaaga a tete c g t g g et e c c c at t g ag c c a a e t e g e g t e c a g t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e t g e tctcccttccgcaagtgcgtgcccgtccccacggaactgatcaagcagaccaagtcgctgaagattgaggaccaggaacagttcc aactggatctgtag

40

30

Human Homologue

Drosophila Gene Hit TBLASTN with ORF1: BcDNA.HL08053 mRNA (AF132557) TBLASTN with ORF1 and BLASTX with AF132557: eukaryotic translation initiation factor 4E binding protein 2 (EIF4EBP2)

(L36056)

45

175

Annotated Drosophila genome Complete gene candidate CG8846 - phas1 translation

initiation factor 4E binding

protein 2

Human homologue of Complete gene candidate CG8846 - 4758260

ref|NP_004087.1|pEIF4EBP2|

eukaryotic translation initiation factor 4E binding

protein 2 (4e-16)

10

5

Putative function EIF4E translation factor binding protein

Confirmation by RNAi Slight reduction in G1 and G2/M indicating fewer cycling

15 cells

WO 01/72774

176

PCT/GB01/01297

Example 59 (Category 5)

Line ID 141/12

Category 2nd chromosome, small imaginal discs

5 Reversion R Map Position 21A/B

Rescue ID BamH1

Rescue Sequence

10 GGCTCTTTCCAAANAGGCAGTTTCTTGNCCCATTTCTTGGATTGCTTTGTAGT GAACTNAATCGTTTTGTTGGTTCCTCTGTCGTCCAGTCTTGTGAAAATTTCGTG ATAATAATGCCTGGATAAATANTTAAGCATTTGGAAAACGGGGGAAAAAGGG CTAAGTTGTGAAGGAAACAATTGAAGTGACCCTTTGTNTATAAACATTCCA CGACGTGTTTCGAAAACAAACAAAGATATGCGGAAACAAAGTGTTAATAAAA 15 GAGCNAAAAATAGAGAGAGAGTGTCGCGATAAGCGGTTGAGCGAGATAGAG AAAATTGTTGATTAAAATGTGTGTCNAAATAAAACATCAAGCCGCTTGAACGA ACAGTCAGTTAGTTGCTTCTGATAATAACCATGGGAAGCGGCNCGTGTGCTTC GCTCCTCGTTACTTATAAAATATTTAAACGTTTGCATTCTTCNTATTTCCGAAT TTTTGCNCCCCTGAANCAACTTNGTTAAACTGCAAATAGCAATGCAAACAAAC 20 GAATAGAAACTGAAATCGACAACNACATGTGAAAATCACAAATCGCA ATTGTCATCCCAAAGATATAGAACAAGCTATAGGGAAGATANAGAATGTAAG TGCCAAACTAAAATAAACAAACAAGAATAACATTTCCACAGGTGTTTTGCATT TCAAATGCATATTTCCGTGGCGGNTACAAATCTTTTCAAAACCG

25 Genomic hit, Accession No. CSC:AC017815

Associated ORF

45

Genscan ORF1 Predicted sequences >17:48:30|GENSCAN_predicted_peptide_2|554_aa MSNKKMFNRTTSVSPGQLHYYHTDFYYSMPDLHKTRKMHGVKRVLVFCLMIVIL PAILIIMPLHLRKTVFADVIYPMAESDIIEIRAGISSIFCSKHTLRMNSNFNAFQLRNK PEIATNRKHIRLKKSMTLPDDTLEYWGFFLLKGAKVRVKFCSRYDGSRILIIHGHR ELNLCGLTDHNKNKLGANYAKGHEQVQVFFEDNVEITEEKGNQDVLMEHENHG GEDLTEDIPQPQVNIPVKQNNSIQPKLIRKKLKKGTIHHGEHDMHAITDLQGSHHT EHILNHHDHSSNSPAHHHNSTAHHREHSSNITNEETSRNHIRNEDEDPDQNSSKTH YSAESPPHRERLKRHNRVAHRNQKRQDLYDTLYKRSKRENVYDRKTIHGGNAIN FTETDESNSVSSFETGLFQCFNGMILLQEFFRPKNECSNPHIMDTSPNKSSMVVHN VIEDGYYYYIFYSDNDHVQNEIHAIFDIYKPTYQYSNMSESQSCLNTTNCTFNISFL SDEIVVVEVPTRDGIEHEEDDITNLISTCHPRSEIYAIFPITVLVLILCCSFL

40 >17:48:30|GENSCAN predicted CDS 2|1665 bp

atgtccaacaaaaagatgttcaacaggactacgtcagtaagtcctggacagttgcattattatcacacggatttctattactcaatgcc ggatttgcataaaacccgcaaaatgcacggcgtgaaaagggtgctggttttctgcctgatgattgtgatactgccggccattcttatc attatgccgctgcattttgcgaaagacggtgtttgccgacgtcatctatcccatggcggagtccgatatcattgagattcgggagga atctcgtcgatcttttgctgaaacacacactgcgtatgaactccaatttcaacgcttttcaactacgtaataagccggaaattgcgacgaaattgcgacgaaatcgcaagcacattaggctgaagaagtcgatgacattgccggatgatacgcttgaatactggggcttcttctttgctgaaaggtgc

caaggtgcgagtgaaattctgctcccgctacgatggatcccgcatcctgatcatccatggtcacagggagcttaatctttgcggtct

177

15

20

10

5

corresponds to CG9524

Annotated *Drosophila* genome genomic segment AE003623

Annotated *Drosophila* genome Complete gene candidate CG9524 - novel His-rich protein

Human homologue of Complete gene candidate

none

Putative function No hon

No homologies which indicate function

25

Confirmation by RNAi Reduced G1 and G2/M peaks indicating fewer cycling cells

178

Example 60 (Category 5)

Line ID

146/2

Category

2nd chromosome, small imaginal discs

5 Reversion NR

Map Position

26B

Rescue ID

EcoR1

Rescue Sequence

10 TTTNATCCAAACTGAGANACTNTTGGCCCCAAAACTGAAAACTCGGACTCGGG CGCGTAAGGGAGTCGGTCNTCGGGAGTCGGTCGTCTTTTGTTGATCTTGAGAC TGAAATTCCAATTGTTGATTTATCTCTCGGCTGCTGCGCCGCGGCTGCGCTGCT GCAGCGCAGTCCCACTCCGATTTGACCAGCGACCAAGTTTATAAAACTTTGAG CCAAAATGCAGCGCGCACAGTTGTTACCAAAACGTTGCACGCGTCGTGGCCC 15

- TCATCAAAACAAAAAAAAAAATATAAGCGAAAATGAAAACGAAATTCGGTTA ACGTCAACAGAAGCTGACAAAAGGCAGAAAAGACCGAAACAAGTTGCAGGG CCAGAGTAAGCCAAGTTAAATGCGAAAGAGAAGCAAGAGNCAAGAAGAAAN AATGGGCACTACATACATATTATAGCCAGCTAATCTGTTGTGCAGTGCGTT TTATCAGCCNNCGAAAAGAAAACGAAAACGAAAAGTCGGTCCAAGTTCGGAC
- 20 TCAAAATCCAAACAGAAGAGACTCCATNCCATCAGAGACACGCGGATCTCAT CTCGGTAATGTCTCAATAAAGTAATCTTAACTGCCGCCGGGAATGTTGGAAA AAGTGAAAATTGAAGCGCTTAACGTGTTTCGAAATACGATACATGAGAAGTCC CAAAAAAAAAA
- 25 Genomic hit, Accession No. CSC:AC019865 Drosophila EST GH19286 (AI388389)

Annotated Drosophila genome genomic segment

AE003481

Annotated *Drosophila* genome Complete gene candidate CG11353 - novel with weak

30

homology to sugar acetylase? CG7525 - tie receptor protein

tyrosine kinase.

Human homologue of Complete gene candidate CG7525- 4e-23 4557869

35

ref|NP 000450.1|pTEK| TEK tyrosine

kinase, endothelial

>gi|464868|sp|Q02763|TIE2

Putative function

Sugar acetylase and receptor tyrosine kinase

40

Confirmation by RNAi

Both gave a reduction in G1 and increase in G2/M peaks

indicating arrest in G2/M

179

Example 61 (Category 5)

Line ID 155/13

Category 2nd chromosome, small imaginal discs

5 Reversion R Map Position 21B

Rescue ID BamH1

Rescue Sequence 1

Rescue ID EcoR1

25 Rescue Sequence 2

NAGA

40

30

35

Genomic hit, Accession No. AC004274

Annotated *Drosophila* genome genomic segment AE003590 Annotated *Drosophila* genome Complete gene candidate CG13693 - novel

45

Human homologue of Complete gene candidate

6e-05 4507659 translocated

180

promoter region (to activated MET oncogene)
>gi|1730009|sp|P12270|TPR_HUMAN POOR MATCH

Putative function No homologies

5

No homologies to indicate function

Confirmation by RNAi Only wild type profiles observed

181

Example 62 (Category 5)

Line ID 162/24

Category 2nd chromosome, small imaginal discs

5 Reversion R Map Position 55C

Rescue ID EcoR1

Rescue Sequence 1

TTTTNTTTCANGGNTCTTTGCNCATAAAANACACGNGCCCTCNTGTCCATTCAC 10 ATTTTACTTGGAGTCGGTAACGTTGAGTTCCGCGTCCGTGCGTTCTGCCTTCCA ATACAAAGTCTGGTGTAATCTACCAAGCATTCCAGTGNGAAAATCAACTCAC ATTGCTCGGTGATCCNTGCGGCGGTATNATCGCACCCGGAATTGCATAAGTTG CGGNGAGCGGAAAGAGTGCACGGATTTNCNGTTATCNAAGGGCCGGCANC NGTGGGGCGCGACGGNAGAGCACGCAGAANAANAATANANTGNNGTGGCG 15 AATTNAAAAATANNATNAAAGAAAATTCGGGCCGCTAATTTTCTTCAAATTT GTGTGCGGTCGCGAAAAACAACGTGTTTTTCNATGGTTGATAATACACACGG ACGGNNCACTCGCGCTCACCCACATAGTCACNAAAGTCGGCGACGTCGACGA CCCNCACNCTCACATANGGACNTTTAATCCCGTNCATNCGTGTAGCGTNCNTA 20 TTTAACCNTNTCTGTCCATCGGAACGCNCGCNTTCTCGCCTTCNTTCTNCTTTA CTTTAATTTCCTATTTNNAAGGGGNAGNCCNATCTTTTTNCCTNTCNNTGCCNT TTAANNTCATCCACANCCTCNCTTTNTCNTTCCTCCNCCTTNTNTTCTTTTCNTC

25

Rescue ID BamH1

Rescue Sequence 2

TTTCTCCTTCCCCCC

AAGNCNCCTTGGCCGNNTTNAACGGNAANTAANCCGGGNCCNCGGGNCNCGA TAATCAGGTCNANCCTTGTGCCTACCACCACCAAATTGAAAAAGAGCNAAGA TTCTCTAAGGCAAAAACTCCCCAATCTGTGGAATTTCCGGAAGCGAGAGCAC 30 ATTCAAAGCTACCAGTTATCAGCGAGCAGCATGTCTAAGCTCAGGAACCTGTT GCCCACAATCTTTGGCGGGAAGGAGGCACAGAATCCGACACCCGTCGAGGGA ACTGCGGAGCCATGGCGCTGCCCTCCACCGCTGGCACGCCCACAGCCTCCTCG 35 GATCTGACCGAATCCGTGCTGCGCGAGCTCAGCGACCCAAACTACAATTCAAT GGATGTGGTGCTTTCNNCCTNTTTTCCGGGCACTCTCAGTAACGTCCAGACAA ACAACACCATGAACGTTCACNGCGCCCAGCAACAGGTGGTCATGAACTTCTCG AATGCCAATAATCTGCACTTCGGCTCCGTCTTCAACTTCAACCAAAACTTGAG CGCCTGCNGCTCNCGAANGGGTTTCACCNGTTCGCANAAGAATCGGTCGCCTC 40 **TCCANACNGT**

Genomic hit, Accession No. CSC:AC012981

Associated ORF

45 Genscan ORFs: ORF2 predicted sequences >18:26:17|GENSCAN predicted peptide 7|1320 aa

182

MEETNNATTIEQQPIALINGQEQVANEQQPSSPTSVATPTSTTSGGTGNATPAFSY DDLFPALPANTSAQSQSGASGSTLARVTSSQKTHIVHVPCKERKSTESEKFGEGES KRICQQITKETGAQIEIASRQVTVPREHFRVILGKGGQRLREIERVTATRINIPSQSD ESEFITIAGTKEGIAQAEQEIRQLSAEQYKKSSDRITVPKVYHPFIVGPYSENLNKLQ EETGARINVPPQQVQKDEIVISGEKDAVAAAKAKVEAIYKDMEKKCSTVSVEVAK PKHRYVIGPKGSTIAEILQLTGVSVEMPPNDSPSETITLRGPQVALGNALTVVYQK SNSVKSVEINAAHWIHKYVFGRKGANMKQLEEDCPNVNVNCLEDKIKLEGDPEN VDRAVAYLSEIIKNYEENFTFEVMTVNPSYYKHIIGKAGANVNRLKDELKVNINIE EREGONNIRIEGPKEGVROAQLELQEKIDKLENEKSKDVIIDRRLHRSIIGAKGEKI 10 REVKDRYRQVTITIPTPQENTDIVKLRGPKEDVDKCHKDLLKLVKEIQESSHIIEVPI FKQFHKFVIGKGGANIKKIRDETQTKIDLPAEGDTNEVIVITGKKENVLEAKERIQK IQNELSDIVTEEVQIPPKYYNSIIGTGGKLISSIMEECGGVSIKFPNSDSKSDKVTIRG PKDDVEKAKVQLLELANERQLASFTAEVRAKQQHHKFLIGKNGASIRKIRDATGA RIIFPSNEDTDKEVITIIGKEESVKKAREQLEAIIKECDEVTEGEVSVDPKHHKHFVA 15 KRGFILHRISEECGGVMISFPRVGINSDKVTIKGAKDCIEAARQRIEEIVADLEAQTT IEVVIPQRHHRTIMGARGFKVQQVTFEFDVQIKFPDRDATEPVEGLTNGGSGENG GENEGQEGEQEVEKEAEQEPVRQCDVIRITGRIEKCEAAKQALLDLIPIEEELSVPF DLHRTIIGPRGANVRQFMSKHDVHVELPPSELKSDVIKVCGTPARVAEAREALVK MIEDYEADRADRELRSFVLQVDVDTEFHSKLIGRHGAVINKLRADHDVIISLPKRD 20 **EPNDRIISITGYQANAEAARDAILEIVGDPETLHREVIEIDKRIHPHLIGQRRRTIRKII** EDNKVNIKFSADDDNPNSIFISGKIEDVENVKELLFGMAEDYERDYLDNVAIAPPTI GAFLTGFWIRCRRCQRERIRHQRRTVGEAKAGQKPDCAQHSVAGGLPALRCWRG SGGLHAYHLRVGPQKLSASGRVSRSPAVAAILQVGVRRGSELEMDQELEQKLELE LELDYRAMSGRAAAVVRTSL

25

30

35

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>18:26:17|GENSCAN_predicted_CDS_7|3963_bp

atggaggaaactaacaacgcaactaccatcgagcagccatcgctctcattaatggccaagagcaggtggccaacgagca gea accate ctege caa ctte agt gee accate agt accaet ag eggeggaa ct gea act gee accee ce ctt taget accaet ag eggeggaa ct gea act gee accee consideration and the consideration according to the consideration accordinggacgacetgtttccggccetgccggccaacacttcggctcaatcgcaatccggagcttccggttcgactctagctcgtgtgacgag ttcccaaaaaactcatattgtgcatgttccctgcaaggagcgcaagtccacggagtcggagaagtttggcgaaggcgagtcgaag egtatttgecageagateaceaaggagaceggageecagategagattgecagteggeaggtgacegtteetegggageactte egatgagagegagtttateaegattgeeggaaceaaggaggtattgeeeaggeegageaggagateegteagetgteageeg agcagtacaagaagtcatcggaccgcatcacggtgcccaaagtttaccatcccttcatcgtgggcccctacagcgagaacctaaa taagetgcaggaggagaccggcgctaggatcaacgtgccgccgcagcaggttcagaaggacgagatcgtcatctcgggcgag aaggacgcggtcgcagcggcaaaggccaaggtggaggccatttacaaggatatggaaaagaagtgctctaccgtcagtgtgga ggtagctaagcccaagcaccgatatgtcattggtccgaagggctccaccatcgccgagattctgcagttgaccggtgtgtctgtag caaaagtccaactcggtcaagtctgtggagatcaatgcggcacattggatccacaagtatgtgttcggtcgcaagggggccaaca tgaagcagctggaggaggactgccccaacgtgaacgtgaattgcctggaagacaagatcaagctggaggagatcccgagaa cgttgacagggctgtagcctacttgtccgaaatcatcaaaaactacgaggagaacttcacattcgaggtgatgacggttaatccttcgtactacaagcacatcatcggtaaggctggagccaacgtaaatcgcctgaaggatgaactgaaggttaacattaacatcgaagag egegagggeeagaacaacateegtategagggteecaaggagggagtaeggeaggegeagettgaattacaagaaaaaateg acaaactggaaaacgaaaatcgaaggatgtgatcatcgaccgccgtctccatcgttctattatcggagctaagggcgagaagatt egegaggtgaaggacegctacegceaggttacaatcacgatacctacgccccaggagaataccgatattgtgaagctgcgcgg acceaaggaggatgtggacaagtgtcacaaggatctgcttaagctggtcaaggagattcaggaatcgtcgcacattatcgaggtg cccatctttaag cagttccaca agttcgttattgg caagggcggcgctaacatcaaaaagatccgcgatgagacccagactaaaattgatctgcctgccgagggtgacaccaacgaagtgatcgtaatcaccggcaagaaggagaacgtgctcgaggcgaaggaacgta

tecaaaagatteaaaacgagettteegacattgteacegaggaggtgeaaatecegeecaagtaetacaacteaateateggeact ggeggeaaacteateteetegateatggaggaatgeggtggttttetateaagtteeeeaacagegaeteeaagagegataaggt cactattcgcggtcccaaggacgatgtggagaaggctaaggttcagctattggagctggccaacggaacggcagctggcttccttt accgccgaggtgcgccaagcagcaacaccacaagttcctgatcggcaagaatggcgcttctatccgtaagattcgcgatgcc actggtgcccgcattatcttcccttcaaacgaggacactgacaaggaagtgatcaccatcattggcaaggaagaaagcgtaaaga aggecegtgageagetggaggegateateaaggagtgegaegaagtaacegaaggtgaggtttetgtegateceaageaceae aagcacttcgtggccaagcgtggcttcatcctgcaccgcatttcggaggagtgcggcggcgtgatgatctccttcccccgtgtcgg catcaactccgataaggtgacgatcaagggtgccaaggactgcattgaagcggcccgccagcgcatcgaggagatcgtcgccg atetggaagegeagaceaceategaggtggtgattceacagegteateategeaceateatgggegeaegtggatttaaggttea acaagtcacctttgagttcgatgtgcagatcaagttccctgatcgtgatgccaccgaacccgtcgagggtctgaccaacggaggc gtcagtgcgatgttatccgaatcacggccagaattgagaagtgcgaggccgccaaacaggctctgcttgatcttatccccatcgag gaggagttgtcggtgcctttcgacctccatcgtaccatcatcggaccgcgggtgccaatgtgcgtcagtttatgtccaagcacgat gtgcacgtagagctgccacctagtgagcttaagtcggatgtgatcaaggtctgcggtacgccggctcgcgtcgccgaggcccgc gaagegetggtgaaaatgattgagggttacgaggetgatagggeegategtgagetgegeteetttgtteteeaggtggaegtaga taeggaatteeattegaageteattggtegteatggegetgtgattaacaagetgegtgeegateaegaegteateatttegetgeet aagegggatgaacccaatgaccgcatcatctctatcaccggctaccaggccaatgcggaggcagcccgcgatgccatcctaga gattgttggcgaccccgagacacttcatcgcgaggttatcgagatcgataaacgcatccacccccacctcattggccaacgccga egeaceattegeaagateategaggataataaggtgaacateaagtteteagetgatgatgacaaceceaattegatetteateagt ggcaagatagaggacgttgagaacgtcaaggagttgctcttcggcatggctgaggactacgagcgtgactacttggataacgtg gegatagegeegeeaacgattggtgeetteetaactgggttetggateegatgeegeaggtgeeagegagaaeggattegteate aaagacgcaccgtgggagaagcaaaagcaggccaaaaacctgactgcgccaacactcagtcgcaggaggacttcccgcact tcgctgctgcgggggctccggtggcctccacgcctatcacctccgtgtggggccccaaaaactaagtgcatcgggccgagtgtc ccgatcgccagcagtagcagcaatactacaagtcggggtgcgccggggatcggagctggagatggaccaggagctggagca gaagetggaactggaacttgaattggattategggeaatgageggeagageggeagtegtgeggacatetetttag

Drosophila Gene Hit BLASTN with rescue sequence 1: dodeca-satellite protein 1 (DDP-1) (AJ238847). TBLASTN with ORF2:dodeca-satellite protein 1 (DDP-1) (AJ238847).

30 *Drosophila* **EST** GH20785 (AI389573), LP07358 (AI294065)

Annotated *Drosophila* genome genomic segment AE003799

Annotated Drosophila genome Complete gene candidate CG5170 - Dpi dodecasattelite

DNA binding protein CG5576 - Bg5 involved in cytoskeleton organization and biogenesis which is putatively a component of the plasma

membrane

Human homologue of Complete gene candidate CG5170- 4885409

ref|NP_005327.1|pHDLBP| high density lipoprotein binding protein >gi|2498434|sp|Q00341|HB

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CG5576- 2e-07 4506539 ref[NP_003795.1|pRIP| UNKNOWN >gi|3426027 (U50062) RIP protein kinase [Homo sapiens]

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Putative function

CG5170: DNA binding protein (homology with Scp160p, a new yeast protein associated with the nuclear membrane and the endoplasmic reticulum, is necessary for maintenance of exact

ploidy)

CG5576: death domain containing protein, possibly involved in

signal transduction

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Confirmation by RNAi

CG5170: Reduced G1 and G2/M peaks indicating fewer

cycling cells and more polyploidy

CG5576: Loss of G1 peak

185

Example 63 (Category 5)

Line ID 40/2

2nd chromosome, small imaginal discs Category

Reversion NR 5 Map Position 39B

> **Rescue ID** BamH1

Rescue Sequence 1

TTTTTGCCTCCGCTTTTTAATTAAAAAAATGTNTGTTTNGCCCTGGAGCTCTCG GTCTGTTAGCGAGCGTTGCCACCTTTCTGCGAGCTGTTGCTGCACACTGCCACT 10 TTACGAACACGCTCTGATAGCGGGACAAAATACGTCAAGGCAGCGACGGTG GGTTACTAGTGAATTTGGAACGGTGGTCTTAAGACGTACTGGTCTTTTATATTT TCATTATTTTTAAATTGTCGCTCATTTACCAATAAACCTTTTTACTTTTTCCTG ATAGTCCGAAGTCAGATCAAATAGGAAGTTTCACAAAAAATTTTCATCCAGAG AAAATACGCCGACGCTATTCGAGTTTTTTGTATTCGTTAACCGGGAAAGAATA 15 GTTCGAATTCGTTCGCACTTTATCGATAGTAGATTGCTATTATGGAGCCCACTA GTAAATTAAATTCCAGACTGATAAAAGCGATCAACTTTTGTTAATGGGT TTAANTCTATAATAATNCTTAGTCCAAATTGTNTCAAAGTAGTCGATAATTTAT AATAACAGTTTTAGATGACCTCTAGGAAATAACTAATTACCCACATNCTTCAA 20 GAAAGTGTTTNCAATTTGTNCTATAATTAAATAACAGTTGTATTAATTATGTTG TNATTGTNACTCATAATACAAATTAAACAATATAAACACACACATAAATAAGAG AATTGGAATATTTTGTCTCAGATTAGATTTNCCAC

Rescue ID EcoR1

25 Rescue Sequence 2

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AACGGGGGCTTCCGCGNCNCCAAAACGCAATNTACCGTTCATGCTGTGAAG CGAAAAAGAGTGGTAGCGCCTACCNTGGCATATGTAGTTAAATCCGTGAAAT AAGTGAATAAGAATATATGTATGTACTTAATTCGAAAACCTTTTCGCCGTCAG CACAACGGGTGAACGAGAGAGCGGAAGTGGAGTTTTTTGTGGCGGGTCGTCT CGCTCGCACCGCAAANGTCGTCCGTGGCTGCGTGTATGGGTGTGTGGAAAAA GCGTCGAGGTGAATGTGGATTTCTAACCACACCAGCATTGCAAAGACATTGAT TGATATTTAAAGCTGCAGCAGCGAACAAAGCAAATCCTAATTTCGGCAAAGTT TAAGAATAACGAGTGACTGGGGCGCGCGCAATAAGATAAAATTGAAGGTTAT CTGTGTGCGTGTGAGTGACCGTNTACCAGTGTGTGTGTGCGANCGTCCATTGT

AAACAAAAACAAGTGTTGTGAGCGGAGAGAAGAAAGGGAAAGAGAGAAAG 35 AGCGAACAGACTGGCGAGAGAAAAAAGAGATGCCACAAANAAAGCAGCGCA CAAAGGAAAGCTGAAAATTTCANTAAATCTGCAAAAGTGAAGAAAACCACAA GAACCCGCAGTCNTGTTAAATAAAACCCAGANTCCAAGAAACNTTAAAAGAA GCAGTGCAAACAACTGGTGCTNTGAATGCGGTTTATTTTGAAAAAAAATGCA

40 ATTCGGTCCGATGGAA

Genomic hit, Accession No. CSC:AC014744

several including LD46342 (AI544109 BLASTN similar to mRNA Drosophila EST L07550)

186

Annotated <i>Drosophila</i> genome genomic segment Annotated <i>Drosophila</i> genome Complete gene candidate	AE003669 CG8678 - novel homology	with ankyrin

Human homologue of Complete gene candidate

CG8678 -gi7661580 B69CEC399B56F35C |ref]NP_056425.1|DKFZP434J 154 protein [Homo sapiens] (2.20E-85)

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Putative function Novel protein with ankyrin domains, unknown function

Confirmation by RNAi Reduced G1 and G2/M indicating fewer cycling cells

187

Example 64 (Category 5)

Line ID 55/12

Category 2nd chromosome, small imaginal discs

5 Reversion NR Map Position 49C

Rescue ID BamH1

Rescue Sequence

10 TCTCATGNTCAGGGGGCCTTTACNATGTCAAAGAGCAAATTGTCCACAGGGCA AAGCCGCGACCGGCAAACGTGGCCCGCCCACAAAGCGAGCATTTTCACATTTT AACTGTCTGGACATTTTGTAAGTTACACCAAGGCAATGATACCAGTAAAAAAG TGATTGTAGGTGTTTTAATATACAATGTCTCTATTACTGCTTTCCTTTATTCAAA 15 AGCCATGTGTAAGTGTAAGTTCTCGATTTCGGCTAGATTTTGAAGTTCTGCCAT TATCAATTAAAAGTCCAGTTCCTCTATAAATTGGTAATAAAATAGCTCTTTACA GCCAAGTATATGTGCAATTTTGTAAGATTAAANGTCCAAATGTTGTGAACCTT TCCTGGCCCTGAATTTTAAAAAACCATTAAATTGGTCCCATTTGACATTAAATG TTCTATGTACATTAATATGACTTTTTGTGGATGGTTTTATAAACAAGCATTACT20 ATATTCTAAAAATCAAGGATAAAGGACNAGCTTTACAGGAGGTAACATTCCTA TTGTACGGCTTTATTTCTTATACCCATAAGAGCATACCACTAGGATCCGTCGA CCTGCAGATCTCTAAAAACTTGCCTTTGCTGGCGTTTTCCATAA

25 Genomic hit, Accession No. AC007085

Associated ORF

Genscan ORF1 predicted sequences >21:54:11|GENSCAN_predicted_peptide_3|108_aa MGLVTAAFKLKRKDIQDRYQHDINRICHTRSTAHTAYAHFAEHLLRRSPRQRFVN GKGAALVLILLVSAARQFSGSTGAYKLGNRVGKVEGEQQEYKLQDRTTHFCGN

>21:54:11|GENSCAN predicted CDS 3|327 bp

atggggetggtaacegeegeetteaagetgaagegeaaggatatecaggacagatatecageatgatattaacegeatetgeeaca cacgtageacgacacaceggegtatgeteattttgeggageatetgttgegacgaagtecaegteaacggtttgteaacggeaa aggtgetgegettgtgeteateeteetgtttetgeggetegacaattttetggetegacaggtgeetacaaactgggtaatagagttg gaaaagtagaaggggaacageaggaatacaaactacaagacagaacaacacatttttgtggcaattaa

spindle/chromosome

Corresponds to CG8732

40 Annotated Drosophila genome genomic segment AE003836
Annotated Drosophila genome Complete gene candidate CG8732 - I(2)44Dea homology to fatty-acid-Coenzyme A ligase, long-chain previously described

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188

		abnormalities in neuroblast squashes
5	Human homologue of Complete gene candidate	1e-171 4758330 ref NP_004448.1 pFACL3 fatty-acid-Coenzyme A ligase, long-chain 3 >gi 4165018 dbj BAA371 and LCFD HUMAN LONG-
10		CHAIN-FATTY-ACIDCOA LIGASE 4 1e-157

Putative function Fatty acid CoA ligase

15 Confirmation by RNAi Only wild type profiles observed

189

Example 65 (Category 5)

Line ID

6/7

Category

2nd chromosome, small imaginal discs

5 Reversion

NR

Map Position

28E

Rescue ID

BamH1

Rescue Sequence 1

10 TATNAATAATCATAGGGCTCTTGCTCTTACGTGTAAGGCCTGCCCCTCTNCCA GTCTATATACAAAGAAAAACACACACACACACTGGCACACTGGTGTTCGCATATG CCAAAGCCGAGTTAATTTCACTTTGTTTAATCTATCGTTTTGGTGTTTTTTGCATTT TTTAACCGCGCAAACGGTATTTGCGCGTTTTTGCGCCTCTTACTTTGCGATTTAT TGCACCGCTTGGCTGTTTTGCAATTTCTATCTTGATTTCATTGGTATTCACG CGTAATGTAATTCTTAGCAGCGTGACCGCGCCGATAACGATAAAAAATACCAC 15 GGGACCAAAAATAAATACCATATGATACCACTTCAGGGAAAAGAAATCCTAT AAAAGGTGTATTTATAATCAAATACTCGGTACTTNTTAATTACTCCAAGAANA ATTAATTTGAAAAAAGGGGTTCCATTATAAAATATATATTAACCGCTTACAC ATAATCCCCAAACAAACAGCGATTGGGATTTAAAAGGTTCTAAGTCCATCAT 20 TATAAAAGATCATTTCCGAAAAACAAAAGAAATAGATTCAAAATTAGGCGAC ATCAGCCGCTGATAANGATCATAAAAATACAGAAGCTNATTCAGCGAATCA GAAANTCCTACTCGCCACTATCCGAAAACNTNGAAAAAAAATGG

25 Rescue ID EcoR1

Rescue Sequence 2

TGAAAGGTAGCAACAACGTTTCCTTGGAAAAAAGCTGTAAATAGTAAACAAAA TTGTCAAGTTAACGAGCCAAAGTTATTAAATAAGGTTCGAGTACGTTGGCATC GGCTGCCCAGGCAGCAAANAAAAACAAAGACGCAGTTCAAGATCAGCTGGAC ACTTAGAAGANTTTAAGAATTGAAGCACATTNNAAAGAAGANAAAACAAGAAC CCCACCAAAAACCCGCGTGCGTTTGTATGTGTGTGTGCCATCAAATTTCCCGC ACTGGGTGAATGTGCNTGCGTGTGTTNTGTGTCATTTAATTTTCCTACCAATAA TCGCCTTCCAAGAAGTGAATACCAGCCGATCCACCGCTAAATCGAAAAAAGTT TNACTCTGGGTTAANTCACTGTTTACGGCTTTTGTGCTATAATTACCTTTCCCG

Genomic hit, Accession No. CSC:AC017934

Associated ORF

40 Genscan partial ORF1 predicted sequences >22:35:21|GENSCAN_predicted_peptide_4|128_aa MGTNSGATAGINNKPVGGATGAGVLVGGGVGGANSSIGGVLSNSLGGGGSGGLS ISGLNAGGQNANVGGMGNVGGDDGGNGMVGGGVNNQQATTPQYTIPGILHFIQ HEWSRFELERSQWDVDRAELQ

TAAGCNGTGGGAANCTAAAANCCAAAACNTNAGAATCCGAATTCCG

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>22:35:21|GENSCAN_predicted_CDS_4|384_bp

190

atgggcaccaattcgggagccaccgctggcataaacaacaagccggttggcggtgcaacaggagccggcgtcettgtaggcg gcggtgtggcggtgccaattcctcgatcggcgtgtcctgtcgaacagcctgggcggtggcggcagcggcggtctgagcatc ageggeteaacgetggtggacagaacgecaatgtgggggaatgggcaacgttggeggggacgacggeggaaacggggtg gtgggcggcggtgtaaataaccagcaggccacaacgccccaatacacaataccgggcatettgcacttcatccagcacgagtgg tegegettegagetggagegateaeagtgggaegtggaeagtggaeagtgcagaattgeag

TBLASTN with ORF1: very weak homology with striatin, Human Homologue

calmodulin-binding protein (STRN) (NM 003162.1)

Drosophila EST several including LD42534 (AI516610), LD03224

AE003619 Annotated *Drosophila* genome genomic segment

Annotated *Drosophila* genome Complete gene candidate CG7392 – novel WD40 family

member

15 Human homologue of Complete gene candidate CG7392- SG2N HUMAN

> CELL-CYCLE NUCLEAR **AUTOANTIGEN SG2NA** (S/G2... 622 e-178 A cellcycle nuclear autoantigen containing WD-40 motifs expressed mainly in S

and G2 phase cells

Putative function WD40 protein a novel nuclear protein mainly expressed in S and

G2 phase cells that was characterized using autoantibodies from a

cancer patient

Confirmation by RNAi Reduction of Glpeak, more polyploidy

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Line ID 103/1

Category 2nd chromosome, small imaginal discs

35 Reversion R **Map Position** 57B

> Rescue ID BamH1

Rescue Sequence 1

40 GATTTCAAAATTAGGCGACATCAGCCCGCTGATAAAGAATCATAAAAAAATACT GAGGCTTATTTTAGCGAGTCAGAGACTCCTACTCGCCAACTATCGAAAACATA GNGAAGATATAGTCGCCAACCGATCTGCCTTCTATAGTGTTGCTTATTGTTGTC CCCTAATCAAATTAATAAAAATCTGCATTAGGCTGCTTCGCCGGCCAGCAACA AATGTTTTACACCTACTGTACTTTTCGCAGAACAGAGATCCAAATGCAGGATC

45 GTTTCCATGACTGTACATTTATTCGGATTAGACATTAAATTACACCCTACAGCT ATACATACTAACAGTGAACACGGCAAATGCTTAGCTAGCATTGGGCCACTTTC GTTGACTGCGAATAAAAATGATTGGCCGATGCCTTTAGCAGATTCCTTTTGAT CGAATTACTCGGATGGCTTGTGTCCACCTCTTACAAGAACTCCTCGCACCA

191

ATCGTTGAGACAGTTGTAGCAATCGGATGCTTGGTTGGAGCTGGCGTGGCACA CCTTCTTCATCCAGTCCTTGGACAGNTTCTTGGNCCTTTTCAGNANCAGGATCT GGTCCCAAACGGNGGAAGGCCTAAAACGAATGGNAATTGATCGGTAGCCCTT GACTGGCATTGGTAATTTGCGCACATGGGNGTCATCGGATTTACACACGCACC ATATCGAATCAGCGTCCTTAAGCGTCAACCGAGGGTTTCCCCAATTCCGGCCA GTTCCGTCACCGACTTGGTTGCCATTGG

Rescue ID EcoR1

Rescue Sequence 2

ATCAAAGCGNCTGGGCCCGTGCATCGCCNCAGCGTTCGTCTTAATTAATTAGT 10 GATTGCAAGCGGGTGCAATTATGCACAAAATTACGGACTAATACAACTGCCC GCTTCGCGCTCTCCATCTCCCTTCCAAATAGTCGTTTGCTCTTCGCACACAA AAGTGTAAACCCTGTGAAAGGTAGCAACAACGTTTCCTTGGAAAAAGCTGTA AATAGTAAACAAAATTGTCAAGTTAACGAGCCAAAGTTATTAAATAAGGTTCG 15 AGTACGTTGGCATCGGCTGCCCAGGCAGCAAAGAAAAACAAAGACGCAGTTC AAGATTCAGCTGGACACTTAGAAGAGTTTAAGAATTGAAGCACATAAAAAAG AGTTTAATTTTCCTACCAATAATCGCCTTTCCAAGACGTGATTACCAGCCGATC 20 CACCGCTTAAAATTGATAAACGTTTTAACTCTTGCGTTACATCAGCTGTTTTAC GGCTTTTTGTGCTATAAGTTACGCTTTTCCCGTAAGCCGTTGGCAACACTAGAA GAGAGAGATAGAGAGTGTGAGCGTGTGAGTGAGCGGGGAATGTGGGGGGCGGT TCCGGTGCGAAAAACGTAGTAGTAGTACATNNAGAGAGTGCGAACGAGAGG GAGGCAGCCAGCGAGTGTCCTGCGACTGCTCCCCCCTTTACCCTCGTCGCTTTT 25 CTATTCGGAAAATTCAATGACCTCATTTGTTTCATGTGCCGAACTTTGCTTTTC TTTCCCAACCTAAAAACGCAAAAAAAAAAACNCCAAACAGGATATACGTNG GAACANTGANCAAACNANTTCGANAAAACCAACAACNANGGACCGTGCCCTG GGGCNCCTGAAAGGCAAACAGCTGGCNNCAAATCCGGAAAAGGATCNGGAA NAACAGGATCNGCGGGCNCAAGGATCNCCGGAACAGGCAAAGGAAACNCCC 30 GGCNCACNGCACAAGCCNCTGAAAAGCAACNTGAACCAATGGGCACCANTTC CGGGANCCACCGCTGGCATTAAA

Genomic hit, Accession No. CSC:AC017934

rest of results as for line 6/7

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Example 66 (Category 5)

Line ID 65/24

Category 2nd chromosome, small imaginal discs

5 Reversion NR Map Position 48A

Rescue ID BamH1

Rescue Sequence

- TACGATTTTTGCANTGCNCCATTTCGTGGCACCCGATTTGTATATATTTTTT
 ATATAACCCACGGATTGCCAACTTTCATTGCCCTTTCACACTCTTATTCGCCAT
 TTATGAACTCTTCTTTGACGATTGGAACGGTTCTTTTTCGCTATTTTCGACTGC
 ACCCGCGCTCTTTTCGCTTCGCTCTCCCCTCTCTACACACCCGCTCTTTATCCT
 TAATTGCTTTTTCTATTTAGCGGAATTGATCGTTCTCAACTTGGTCGCCATTGC

 AGCTCCACAGGCGAAAAAAATCGGTGGAAATGCCAATACAGGTGCACGGCGAG

25 Rescue ID EcoR1

Rescue Sequence 2

- 30 ATTAATAAAAACTGCAATAATGTCAAAAAATCTAATTGAGGCAACAAATTAN CAAAGCCATNAAAGCAGGCTGCACTGCGAGAAAATTGTGCCTTTCCACAGAT CTTCTGCTGCAAAGCNAAAGAANGTAAGCAAGTCGGCCANTTTATTNCATTCT TCTCATCTCTTCTTCGCGAATTGGCGCNTANCACTTACAATAATTNATATNA CTTCTTAAATTTCAAANTCCCTTTCNTGAACGGANCTTTTAACGGAAAACAAA
- 35 GCGGGTAAACTAACTAAACTAAATTANAANTGTANGTATAAATGAACC GAACTCGCTTTAGATATNATGCGTTTCACTAACANATTANAACAAACTTTGAA GCTGTANTGTCAGGTTGTTATTNCGTTCACCANATGTAGACTGNCCGNNAATT TNACCTTTCCCATANTCTGTTCTTAANTGTNTTGTTTTTTCCCAATNNTTTGATC ATNCNTTGGTNAATNANCTNAACGGCCCAAAGTNAATGAATTCCANTCACGTC
- 40 CACTGGCTCTGGTTCNATANTTAATNGGCTGTTTCTTACTTCCCTTAACCCTAA CATCTNTTAATCACCTGTGCCATNTGTTTGTGTGTGTGTGAACGAATGAGAAA AAAAA

Annotated Drosophila genome genomic segment

AE003825

193

Annotated *Drosophila* genome Complete gene candidate CG9005 - novel putative cell adhesion

Human homologue of Complete gene candidate CG9005- Ensembl predicted gene

ENSP00000006008

Gene:ENSG00000005238

Clone:AC004472

Contig:AC004472.00001 6.00E-38 (KIAA1539 protein AB040972) and AK022837 Homo sapiens cDNA

FLJ12775 4e-33

Putative function Putative cell adhesion protein

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Confirmation by RNAi Reduced G2/M peak

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Example 67 (Category 5)

Line ID

74/3

Category

2nd chromosome, small imaginal discs

5 Reversion

NR

Map Position

47A

Rescue ID

EcoR1

Rescue Sequence

10 GCACAGAATGGCNCCTTCACGACAAAAGATCTNCNAATTAGGATGATGCAGA AGGAGGACACGCTTTTCATTATCTGGTTGCCACCTAATTTAAGTTCCACATCAA GAAAAGCCTGTCTATAAAAACACGATAACGTTTTTGCTAATCTCAAGACAATG TTAAATATAATTGGAGAAAGTATTGAATATGAATATCACAAAAATTGTTTAGG 15 GTCTCTACGTGGTAAATAGTATTTGGCATAGACAGTGAGATGTGAGTCGTACG TACTAATTAATAAAGTTGTTCAARAGAACCTCATATACTGTAAGTGACAACGA ACGAAGCTGACAACTCTGCTTGCACATATTTGGCGGAGTTCGAAAATATCATC GCATTGGTATTGTTTTGTNTCCACCNTGGGGCGAGATTTTGTTGTTGCTTTAC TTTGCTTGTTTTTCNCCACAAANCGAACCATAATGTTCGAAATGGTAAAATTA 20 CCGTGCCAACAAGCTCTCTCTCCCCACTCCGAAACTCTCTCATCTCCTTG CAATTGTTTAAGGTGTGCAAGGAAATGAAAAATGTCCCGGCTGTGTTNCCATG CATTCCCCTTCAAAGCCAATTATNTTTGTGCCTCTCCAACNTTTTTGATCGGNN TGATTTTTTTGGCTCCCCNTANTCCCCCCCCCTTTCNCCCATTCCGGGTTANAT TATTNTNCCAATTTTCCTATTTTACGGTCCCNGTTCCCTGGAAATANTTCCTNC

25 AATCNCCGCTCCATNTCNCCATNTTTGACAGATTTTC

Annotated Drosophila genome genomic segment

AE003829

Annotated Drosophila genome Complete gene candidate CG12052 lola -a specific RNA

polymerase II transcription factor involved in axon

guidance

Human homologue of Complete gene candidate

1e-09 3789797 (AF059569)

actin binding protein

MAYVEN [Homo sapiens]

Putative function

lola-like specific RNA polymerase II transcription factor

Confirmation by RNAi

Almost no G1 peak and increase in G2/M peak indicating

arrest in G2/M

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PCT/GB01/01297

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Example 68 (Category 5)

Line ID 79/7

Category 2nd chromosome, small imaginal discs

5 Reversion R Map Position 55B

Rescue ID BamH1

Rescue Sequence 1

10 GTCTCATGCACCCTGGCCCTNAGCTGCATAAGTGTAAGTGTGTGTCTGTGTGC GAGTGTGGGTAGGCGGCGCAACTATCTCGCTTGCTCTTGCGTCCGGGGTTAT CGGTAGCTTCTTCTAGGCTGAGTGCATTTCGTTGAATCGTGGATGTTGAAAGTT GTCTAATTTCCGAACTATTGATTTTTCCCCTTCCCCGTCAAGAAACTGCATTGT TGCTTCTTGAAGACCAGTTTTGGTAACATCAGGAGAAATGGAAAGGAGCGAGT

- 15 GAGTCGGTGAGTAAGTGAGTGAGCGATGCGAGCGACAAAATCAACAACAACA ACAACAACGGTCAAAACGAGTTCCAACGAAAGTTGCAACACTCTCAACAATT TGAGCAGCTCCGTTTGTTGTTATTGCATTACTCAATCGGGAAGACTCTACACTC GACGGAATAGTGTGCTCGTCTGAAATTTATCNATTTCCATTCCTTTCTTT TTGGGCCAAACAATGGCNTCGGCAANCGTTCGTGGAAAACCGCAGGAACCAC

25 **Rescue ID** EcoR1

Rescue Sequence 2

 $NGGNGTCTCATGCACCCTGGCCCTNAGCTGCATAAGTGTAAGTGTGTGNCTGT\\GTGCGAGTGTGGGTAGGCGGCGGCAACTATCTCGCTTGCTCTTGCGTCCGGGG\\TTATCGGTAGCTTCTTCTAGGCTGAGTGCATTTCGTTGAATCGTGGATGTTGAA$

- 30 AGTTGTCTAATTTCCGAACTATTGATTTTTCCCCTTCCCCGTCAAGAAACTGCA TTGTTGCTTCTTGAAGACCAGTTTTGGTAACATCAGGAGAATGGAAAGGAGCG AGTGAGTCGGTGAGTAAGTGAGTGAGCGATGCGAGCGACAAAATCAACAACA ACAACAACAACGGTTCAAAACGAGTTCCAACGAAAGTTGCAACACTCTCAAC AATTTGAGCAGCTCCGTTTGTTGTTATTGCATTACTCAATCGGGAAGAACTCTA
- 40 GTNCACATACACTTGTCTTTTTNCCACACACTTTCCTAATCATNNTA

Genomic hit, Accession No. AC004296

Associated ORF

45 Genscan: ORF2 predicted sequences >15:31:31|GENSCAN_predicted_peptide_3|109_aa MVTSFRHLRDEKSFTDVTLACEGQTCKAHKMVLSACSPYFKALLEENPSKHPIIIL

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4.00E-49 (potental cell division GTP binding protein

1: ENST00000051207

196

KDVSYIHLQAILEFMYAGEVNVSQEQLPAFLKTADRLKVKGLAETPSSIKREG

>15:31:31|GENSCAN predicted CDS 3|330 bp

atggtgacctcgttccgtcacctgcgcgacgagaagagcttcacagatgtaacactcgcctgcgagggccaaacctgcaaagcccacaaaatggtgctttccgcttgcagtccctactttaaagcgctactggaggagaacccatcgaagcatccgatcattatcctgaaagatgtctcctacattcacctacaggctatactggagttcatgtacgccggtgaggtgaacgtgtcccaggaacaattgccagcatttcttaagaccgccgatcgcctcaaagtgaaaggcctcgcagagaacacccagttcgataaagcgggaaggttga

Drosophila Gene Hit TBLASTN with ORF2: several zinc finger proteins including Broad-Complex mRNA for BRcore-Z2 protein (X54665)

Human Homologue TBLASTN with ORF2: kelch (Drosophila)-like 2 (Mayven actin

binding protein) (KLHL2) (AF059569)

Annotated Drosophila genome genomic segment AE003800 Annotated Drosophila genome Complete gene candidate CG5738-15 lolal. lola-like putative kelch-like putative specific RNA polymerase II transcription factor known to affect disc morphology 20 or could be CG10914 - novel unknown CG5738- 9e-09 3789797 Human homologue of Complete gene candidate 25 (AF059569) actin binding protein MAYVEN [Homo sapiens] CG10914- predicted gene ENSP00000051207 30 Gene:ENSG00000047313 Clone:AC068261 Contig:AC068261.00019

Putative function CG5738: lola like specific RNA polymersae II transcription factor, CG10914: Possible GTP binding protein

Confirmation by RNAi Both show marked reduction in G1 to G2/M ratio

197

PCT/GB01/01297

Example 69 (Category 5)

Line ID 80/2, 81/8

Category 2nd chromosome, small imaginal discs

5 Reversion R Map Position 57D/E

Rescue ID BamH1

Rescue Sequence 1

10 CANTTTCAGAGGCCATAGNCCTTCACAAAATTCNCCATCTCTGCCCGGCATCC GTGCTTGAAAATGGTGCCAATGCGTCGTGGAGAATCTGCTGCACTCGATGGTC TGCAAAATTGCACATTTATTAGATTTAATAAATTTTTCAACTGTCCGCGANCAC GTTTGCTCGTGTTGAATTTCGAGTACAAAATTAGTGCGACTGTTGGATTGCATT GAAATGCCAAAAATCGGTGTGACCATTTCGAAGTCCCCACAGGCTCATGACTT TCGCGGTTCACCAAATCCAAATAACGCAAGCTGGTCACGCTGTCAAACATCGG 15 TGACGGAATGGTGACGACAAACAATTTGCTTAAAAACTTTCTTGCGGCCGT AAAAATGCGCAAGCAGCCTGGCAGCGCAACGCACGTACACGTAATTGGAACA AATGTTTGCTGAACCACAACCGCCCACTAAATGTTANCCGCCAAGTCTTTTCC CCCGCCGCCGCCTCNTCNTCNCCGGATTATTTGGTTTACAATTTGCTTAC 20 ACAAGTGCAATCGTCGATAGCGCTTCATTTTGGAGTAACAAGTAATATTTTGC GCCGTACTGCTGTTCGCCGTATCAGACAGAAGGTTGGTATCAGTTCGACGCAG CTTGTGACGGTATTGCATACGCGGCGAAACGCCCACGTGAAAACGGATCGCA GTTCTCGAAAACTCNGGATAAAAA

25 Rescue ID EcoR1

Rescue Sequence 2

TGGGGTCTCANGCCCCGACGGCCATATTTTAACACAAGATTCNNCANCTCTGC AGGGCATCCGTGCTTGAAAATGGTGCCAATGCGTCGTGGAGAATCTGCTGCAC TCGATGGTCTGCAAAATTGCACATTTATTAGATTTAATAAATTTTTCAACTGTC 30 CGCGAGCACGTTTGCTCGGTGTTGAATTTCGAGTACAAAATTAGTGCGACTGT TGGATTGCATTGAAATGCCAAAAATCGGTGTGACCATTTCGAAGTCCCCACAG GCTCATGACTTTCGCGGTTCACCAAATCCAAATAACGCAAGCTGGTCACGCTG TCAAACATCGGTGACGGAATGGTGACGACACAAACAATTTGCTTAAAAAACTTT CTTGCGGCCGTAAAAATGCGCAAGCAGCCTGGCAGCGCAACGCACGTACACG TAATTGGAACAATGTTTGCTGAACCACAACCGCCCACTAAATGTTAGCGCCA 35 ACTNCTTTTCCCCGCCGCCGCCGGTCGTCNTCNTCCCGGATTATTTTGTTTACA ATTTGCTTACACAAGTGCAATCGTCGATAGCGCTTCATTTTGGAGTAACAAGT AGTATTTTGCGCCGTACTGCTGTTCGCCGTATCANACAGAAGGTTGGTATCAG TTCGACGCAGCTTGTGACGGTATGCATACGCGGGGAAACGCCACGTGAAAAC 40 GGATCGCAGTNCTCGAAACTCNGGATAAAAGAAAAAGTAGGCTGAATG

Genomic hit, Accession No. AC007175

Associated ORF

45 Genscan: ORF2 predicted sequences >16:09:09|GENSCAN_predicted_peptide_3|2497_aa MNEGNSAGGGHEGLSPAPPAVPDRVTPHSTEISVAPANSTSTTVRAAGSVGAALP

ATRHHOHIATOVKGIASSSSKOOKOLASAOLPVPLSPLPOQOQOTAEATAAAAAP AHSNVSVSSSTIEASVLPPOAKRORLDDNEDRTSAASIVGPAESSNIVSSLLPASVA SSSEVGGLSSTALODLNALKKRILOOKLQILRNLKERHLENVSEYFYLQNGGSMM DYPAWRKKTPTPOFISYSNANRIDQLIHEDKPSTSAAAAAAQNQKYTTQQTDSVE SSLVSGIGTGATKGAPLDGNISNSTVKTNTQSQVPSKIGSFTESTPAATESNSSTTVP GTATSGAATSTSATSAEASGNVLAVEAEIKIPAVGATPVAISTKLPAAVVQLTQQG GTPLLPCNTSAGSTALRRPOGONNASSGSAAASGGGGSLTPTPLYTGNGPAALGG SGGLTPGTPTSGSLLSPALGGGSGTPNSAAQEFSFKAKQEVYVMQRISELQREGL WTERRLPKLQEPSRPKAHWDYLLEEMVWLAADFAQERKWKKNAAKKCAKMV 10 OKYFODKATAAORAEKAOELOLKRVASFIAREVKSFWSNVEKLVEYKHOTKIEE KRKOALDOHLSFIVDOTEKFSOOLVEGMNKSVADTPSLNSSRLTSPKRESDDDFR PESGSEDDEETIAKAEEDAADVKEEVTALAKESEMDFDDFLNDLPPGYLENRDKL MKEEOSSAIKTETPDDSDDSEFEAKEASDDDENTISKOEEAEOEIDHKKEIDELEA DNDLSVEQLLAKYKSEQPPSPKRRKLAPRDPELDSDDDSTAVDSTEESEDAATED 15 EEDLSTVKTDTDMEEQDEQEDGLKSLMADADATSGAAGSGSTAGASGNKDDML NDAAALAESLOPKGNTLSSTNVVTPVPFLLKHSLREYQHIGLDWLVTMNERKLN GILADEMGLGKTIOTIALLAHLACAKGNWGPHLIVVPSSVMLNWEMEFKKWCPG-FKILTYYGSQKERKLKRVGWTKPNAFHVCITSYKLVVQDQQSFRRKKWKYLILD EAQNIKNFKSQRWQLLLNFSTERRLLLTGTPLQNDLMELWSLMHFLMPYVFSSHR 20 EFKEWFSNPMTGMIEGNMEYNETLITRLHKVIRPFLLRRLKKEVEKOMPKKYEHV ITCRLSNRORYLYEDFMSRAKTRETLOTGNLLSVINVLMQLRKVCNHPNMFEARP TISPFOMDGITFHTPRLVCDIMEYDPFTQINLETLNLLLLHLEQTMTAYVSHKSRLL APPRKLIEDIDTAPLPAPRCPNGKYRFHIRVRSAELAORIKLNAVKVGASPAMRLE GSKIMPMRNLLPSGRVLKRVSASINPVNMALKPVVINSVVTTTSSSTTASSPTGAL 25 SVLSNSKLLGARSQINAPTPAKVAKTMQDGKPFFYLTPATNSGAAGARLTLTSKT TASASTTTSRTTVTASTTSGOOLIRDPIVKDLATHVKSTVOKOSIANGKTEPEEETE AEDPYKVOELIOMRKEORLAALKRMAMINRRRTDATPIYGEDCREAIORCMQAT RSLKRSTWQTRGYANCCTAMAHRNGWSLNHLLKSFEERCADLKPVFANFVIYVP SVCAPRIRRYVQNLSSTHWQHEQRIENIVDQALRPKLALLHPIISEMTTKFPDPRLI OYDCGKLOTMDRLLROLKVNGHRVLIFTOMTKMLDVLEAFLNYHGHIYLRLDGS 30 TRVEQRQILMERFNGDKRIFCFILSTRSGGVGINLTGADTVIFYDSDWNPTMDAQA ODRCHRIGOTRDVHIYRLVSERTIEVNILKKANQKRMLSDMAIEGGNFTTTYFKSS TIKDLFTMEQSEQDESSQEKSENKDRIVATTTLSDTPSTVVETEKQSLRAFEHALA AAEDEQDVQATKTAKAEVAADLAEFDENIPIATEDPNAEGGPQVELSKADLEMQ NLVKQLSPIERYAMRFVEETGAAWTAEQLRAAEAELEAQKREWEANRLAAMHK 35 EEELLKQETEAEEMLTYSRKDSSNQVNTKTDSNSNKRRLVRENRRNSAOKLSRSV SSHSTGSNNKNSKSATTRGNSONSLNOTVPVGSGISRVNRTGAGVSSSSRGKSNST KSTGKGTDAAPQVRRQTRLHSLGAVNMASARTPPTRKTTRTALAASAAASTLED ASLIVEERPKROSANIAMSKMMKTPFKONVPSNISIKTTPPKRGRRDSVAAAATRS 40 KLLERRATIAAPLKHMDDESDQDEEEQEEQESEEDTEGEEANATVDDDEEGEEEL ASLDEETIOTGSOTNDEEDDDEEEVGEEGMVDIDTEDSEADVKSSSTYGTAADGK PEEAESLDGWDAHDQVQDTTMTSSTYYNVSEESDTDEHHDSKAEAKEPPQNSDK SDESEAVGHTPRTRSRGTVKINLWTLDVSPVANALNKSSANRSLKKAPRTESTPK ESQSEPRRKITQPKLPKKEETNNKSNSNIGTLHRWISKSPRVMLRSTPVTAASASSS 45 AAVSGVSGGNASSSGTAR

>16:09:09|GENSCAN_predicted_CDS_3|7494_bp atgaatgaaggtaattcagcaggaggggggatgaagggctcagcccggccctcctgctgtgccagaccgcgtaactccaca

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tteaaeggaaattteagttgeeeeggeaattetaeaageaeaacagtaegageaggageaggageageettgeeggee accegceatcaccaacatatagegacceaagtgaagggaategceagcagcagcagcaacaacagaagcaactggccagtg gaggacaggacgagtgccgccagcattgttggaccagccgagagcagcaacattgtaagctccctgctaccagcgtcggtggc ctccagcagcgaggtcggcgggctttcttctacggccctgcaggacttgaatgccctcaagaagcgcatactccagcagaaattgcege g t g g c g caa g a a g a cacca a cece g cag t t cat cag cta cag caa t g c g a a t c g t a t a g a t cag cta t a g a t cag cta cag caa g a t a cac g agtcagtggcatcggtactggagcgacaaaaggagcgccattggatggcaatatcagcaatagtactgtgaaaacgaatacgcaa tetea ag ttee aag caa ag at tege ag at tea aag aat caa ag caa ag caa ag taa caa ag ttee aag ta caa ag taa caa ag taa agacagctacaagtggcgccgcaaccagcacatcagctacttcggccgaggctagtggtaatgtcctggcagtggaagcagaaatc aaaatcccagctgttggggccacaccagtggccatttccaccaagcttcccgctgccgtcgtccagctaacgcaacaaggtggca ccctttattgcctgcaatacatccgccgggtccacggcgttcgtcgtccccaaggtcagaacaatgcctcaagcggatccgcegeggeatetggaggeggaggaagceteacaccacaccgetetacactggeaatggceeggeegetetgggeggtagegga caggagt to tott tta aggccaag cag agg t g tat g tag cag c g tat at c gaac tac ag ag ag agg g at tat g g ac t g ag c g agggegeetgeeeagetgeagageeeageegeeeaaggggeattgggactatettetegaggagatggtetggetggeggea gattttgcacaggaacgcaagtggaagaaaaacgcggccaagaagtgtgccaagatggtgcagaagtatttccaggacaaggccaceget geceagegggeggaaa aggeeceag agget geaget a agget get teet that the cacegeg agget gaag agget the contract of the contract of the caceger grant and the caceger grant gcagetttattgtagaccagacagaaaagttctcacagcaattggtagagggaatgaacaagagtgtggcggatacgcccagtctta attetag cegtetaa eateg cegaaa egggag teegat gat gat tetege cet gag tet gegtetag at gat gat gag agac tateger than the contraction of the contractcgag caggagatagaccacaaaaaggagatcgatgaactggaggcagacaatgatctctcagtggagcagttgttggcgaaatacaagtotgaacaacotcotagtoccaagogacgaaagttagogocgogtgatoctgagotggactotgatgatgattogaoggo gaacaggatgaacaggaggacggtcttaagagtctaatggcggacgctgatgcaacaagtggtgctgctggcagcggaagcac ggetggggcaageggcaacaaggatgatatgetgaacgacgetgeegecetggeegagageeteeageeeaagggtaatace ttgtcctcaaccaatgtggttactcctgtgcccttcctgctaaagcactccttgcgtgagtaccagcacatcgggctcgattggctggt ccaccttgcctgcgcaaagggcaactggggacctcatctcattgtggtgccttcgtctgtgatgctcaattgggaaatggagttcaa gaagtggtgccccggctttaaaatactcacctactacggctcccagaaggagcgcaagctaaaacgcgtaggttggaccaagcc cctggatgaagcgcagaacattaagaactttaagtcccagcgctggcagttgctacttaacttttccacagagaggcgtctgttatta actggaaccccactacagaacgatetgatggagctgtggtccctgatgcacttccttatgccatatgtgttctcatcgcaccgcgagt ttaaggaatggttctcgaacccaatgactggcatgattgagggcaacatggagtacaacgagactttaattactcgtctgcacaagg tgattcgtccgttcctacttcgacgcctcaaaaaggaggtggaaaaacagatgcccaagaagtacgagcatgttataacgtgtcgt ctgtcgaatcgccagcgctatttatatgaggacttcatgagccgccaaaactcgtgagactctgcaaacgggaaacttgttgag cgtgataaatgtactgatgcagttgcgaaaagtgtgcaatcatccgaacatgtttgaagcgcgtcctacgatctcgccatttcaaatg gatategataeggeteeattgeeageteeeegttgteeaaatggeaaataeegettteatateegagttegtagegetgaaetggeg gctaccaagtggaagagtgctgaaaagggtcagtgcttcgatcaaccctgtgaatatggctttgaaaccagtggtgatcaatagtgt ggtgacaacaacatcatcatcgaccacagcatcttctcctactggagctttaagcgtgctgagcaactccaagttgctgggtgcac

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Drosophila Gene Hit TBLASTN with ORF2: brahma protein (M85049) and imitation-SWI protein (ISWI) (L27127) and chromodomain-helicase-DNAbinding (CHD-1)

45 Human Homologue

BLASTX with EST TBLASTN with ORF2: Snf2-related CBP activator protein (SRCAP) (AF143946) and SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) (NM 003072.1)

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Drosophila EST several including SD07794 (AI534784), LD34465 (AA990657) Annotated Drosophila genome genomic segment AE003453 Annotated Drosophila genome Complete gene candidate CG9696 - domino an enzyme involved in DNA repair 5 homology to snf2 family helicases Human homologue of Complete gene candidate CG9696- gi4557447 10 416409C913D6A935 |ref|NP_001261.1| chromodomain helicase DNA binding protein 1 [Homo sapiens] (1.90E-85 15 snf2 helicase family member protein that contains a Putative function chromodomain, which occurs in proteins that are implicated in chromatin compaction, and an SNF2/SWI2-like helicase domain, which occurs in proteins 20 that are believed to activate transcription by counteracting the repressive effects of chromatin structure Confirmation by RNAi Loss of G1, peak, increae in G2M indicating arrest in G2/M

202

Example 70 (Category 5)

99/31 Line ID

Category 2nd chromosome, small imaginal discs

5 Reversion NR **Map Position** 53E

> **Rescue ID** EcoR1

Rescue Sequence 1

10 AAGGCCCGACCAGAAACGAAATTTTCGGCGCGTNTTTTAAAATGCGCGGTAA TTGTGTGTTCGCCTGGCTTTGCCTTTTAATTTTTATTTACCTGCATCCGATTCG GTATTTGAAACAGCCGTTGAGTCTCCTTTGGCTTTTTTATCAGCGACGTCATCA GTGGCGGCAGAAGCAGAAGCGTCGACAGCGGCGGGGGATTCGGCTGCATCTT TGGAGCCCCTTTCCGGCTGTGCCCCCACGGCTTTCGCCACCCCCGCAGTAACC 15 GATGCATTTTCCACATCGCTTACCTTATCGGCGGCATTTTCTTTGGCTGCCGTT TCTGCCGCTTTGTTAGCATCCTTTTCGTGCGGCGANGGCATGGAAAGATACAA ATCAGAATTGGATTACACTTGCTAATTTTTTGGCGGNCAATACAATGGTTCGG TGCGCCTATTCTTTTTAATCGAATCGCAATTGAGTGTNAATTAAGTCTCCGCA 20 ATGCAATTTGTGTATCTGTCTCCCCCGANCGAACAACGATNGAAAAAGGAA CCAGAAATAAAANAGGNAATGAAAAAACACATTGCAATCTATAAGGCCACAC ACACACATATCATCCCGTCTACCANTCCATCGGATTCGANCCACANAANCCAT NTTTATACCNCAACGAACGNGGAAAAAACNATATCNGNAATTACCCCCCGAA AATTGTTGCCNCTTTTACCAAATATTTACAACCNCCGTTCATTCACTCCTGGA ACATTCCNGGCTTTCCCAATTTTCNCCTTTACTACAATTTCAATGGTTTCTTTTT 25 **CCTCAC**

Rescue ID BamH1

Rescue Sequence 2

30 CCTNAAATGTNGCGCTGGGNCCTAAANCGTCNCTCCTTGTGTCTCTCTTGTTTA CCGCGCTATGCTGATGTTGGCATGTTCGATCCCCCTCCGTGTCGATGTTTA CCTTCCTTGGCTTTTGTTCATGCTAAATCCTTTAAATGGGGTTCTGCGTAGTTT AATGCCGAGGTACAGCAAAACTTCAATATTCATGTTCCCTTGCGCTCCCAAAC GAAATTAGCATTGGACGTCCCAAGGTTGAAGACATTTNATTATTTTAACATCT 35 TTTTNATTTATTACATTTGAACTCTTACAAGTAATAATAATTACAATTAATAT TATAGCTGCAGCGGACAAAAAGGAGAAATCCCCCTCGCCGGTAATAAAGAAT CCAACAATAAGGATGCTNAAAANGAAGAAAACCCNAAAAAGGAGAAGAAAA ATCGGAANAAGGNGATGAGCCNGAAGATGAGGNNGATGAGAAAGCTAGCGA TGAAGAGAGCGAGAAGAAGAAANCGANATGAGATGCAGAGGACAGATAAAG 40 GATGCCACNGATGAATCCAAGCCAAAATCGGGAGCCGATAAGCCCAAGAAAC

Genomic hit, Accession No. CSC:AC020063

TGAGCCCAAGGCCAAGAATGGCAAGGTGGNT

203

Genscan ORF1 predicted sequences >16:48:25|GENSCAN_predicted peptide_1|722 aa MPSPHEKDANKAAETAAKENAADKVSDVENASVTAGVAKAVGAQPERGSKDA AESPAAVDASASAATDDVADKKAKGDSTAVSNTESDAAAADKKEKSPSPVIKKS NNKDAKKEDNSEKDEENSEDGDEPEDEADEKASDEESEKKKPKLDAEDKIKDAT 5 DESKPKSGADKPKKPEPKAKNGKVAKEEDDDEEDEDDEDAEDDDGDENDGLDK NNEVAEDDENVVALAEIDRINENINKTRVDGLQTLHAICFGAQGKNNVVKKNLRS FAGFEFAKDSAEYNKKLEAIKKVDNKGLRSICEILTLDRKGSKNETVLRVLKFLM EPDESLCLEOGDEEEEDAEDEDLDEDEEDPPSEEDKKRKSGKSSGGAGRGSARN STGRPRRATAGKKMSAYVDFSSSDDSEQKVAVPKRRRNDDSESGSDYNPSANSD 10 SDGGRGGGAGAAGRKVPSRGGRGRPARKSRRRNSDSEEEEESEVSDADSDVPKR KRGSVGKRGRPAAPASAGRRGRGRGAASRKRKDSDSEDEEVSEDEEEEDVSDFA SDQSEVCKFNLISSIWCFIKYMPIFQEERPKKSKKPITPAKNSKANNKSKPAGKADS RSKKSKKESSEEDDDVDDKDESDEDEPLTKKGKQAFPTDEQIRGYVKEILDKANL EEITMKTVCKOVYAKYPDFDLTDKKDFIKATVKADGVQDLDGSPELIPRGRTTVT 15 **IWLICCCNNQIFGET**

>16:48:25|GENSCAN_predicted_CDS_1|2169_bp

atgccatcgccgcacgaaaaggatgctaacaaagcggcagaaacggcagccaaagaaaatgccgccgataaggtaagcgatg tggaaaatgcatcggttactgcggggtggcgaaagccgtgggggcacagccggaaaggggctccaaagatgcagccgaatc 20 $\verb|ccccgccgctgtcgacgcctctgcctctgccgccactgatgacgtcgetgataaaaaagccaaaggagactcaacggctgtttca||$ aataccgaatcggatgcagctgcagcggacaaaaaggagaaatccccctcgccggtaataaagaagtccaacaataaggatgc taaaaaggaggacaactccgaaaaggacgaggagaactcggaagacggcgatgagccagaagatgaggctgatgagaaagc tagcgatgaagagagagagaagaagaaaccgaaattagatgcagaggacaagataaaggatgccactgatgagtccaagcca aaatcgggagccgataagcccaagaaacctgagcccaaggccaagatggcaaggtggctaaggaggaggacgacgacga 25 agaggacgaggatgatgaggatgccgaagatgacgatggagacgatggacaggatggcctggacaagaacaacgaggtggccg aggatgatgatgatgtegtegtetetegeegagattgategeattaatgagaatateaacaagaetegtgtagatggtetgeaaacat tgcatgcaatctgctttggcgcccaaggcaagaacaatgtggtcaagaagaacttgcgatcctttgccggtttcgagtttgccaagg atteageggagtaeaaeaaaaagetggaggeeateaaaaaggtggataataagggeetgegeageatetgegagateettaeeetegategeaaggeageagaagaactgteettegagtgeteaaatteetaatggaaceggaegagtegetttgettggagea 30 gggtgatgaggaggaggaggaggatgcegaggacgaggatetggatgaagatgaggaggacccgcccagtgaagaggaca agaagegeaagageggaaagtetageggeggegetggeagaggetetgeaegeaattecaeeggaegtecaaggegegega cggcaggaaagaaaatgtccgcctatgtagatttctccagctctgacgatagcgagcagaaagttgcagttcccaaaaggagacg aggtegeaaagteecaageegeggtggaegeggtegteetgegegeaaaagtegeagaagaaactetgatteegaggaagaa gaggaatcggaagtttccgatgccgatagtgatgtcccaaaacgtaaacgtggttccgtgggtaaacgtggacgaccggcagct35 gtgttttatcaagtatatgccaatttttcaggaggaacgtcccaaaaagagcaagaagcccattacgcctgcgaaaaatagcaaag 40 gtcgatgacaaagatgaatccgacgaggatgagccactaaccaaaaagggcaaacaggcattcccaacggatgaacaaatacg eggatatgteaaagagattetggataaagceaatettgaggagattaegatgaaaacegtgtgeaaacaagtttatgeaaaatatee agaetttgacetaacagacaagaaagaettcatcaaggegacagtgaaageggaeggagttcaggatttggatggtagtcccga

45 **Human Homologue** TBLASTN with ORF1: poor homology with DEK gene (D6S231E) (NM_003472.1)

Drosophila EST several including LD33301 (AA979048)

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		a genome genomic segment a genome Complete gene candidat	AE003805 te CG5935 - EG:EG0003.6 - novel with weak homology to	
5			DEK oncogene CG8648 - EG:EG0003.3 - novel XPG/ flap endonuclease-like, DNA repair?	
10	Human nomologue o	f Complete gene candidate	CG5935- 1e-17 4503249 ref[NP_003463.1 pD6S231E DEK gene	
		,	>gi 544150 sp P35659 DEK_H UMAN DEK PROTEIN >gi 284375	
15			CG8648- 4758356 ref NP_004102.1 pFEN1 flap structure-specific	
20			endonuclease 1; MATURATION FACTOR 1 (MF1); DNase IV; RAD2_HUMAN(aa)	
25	Putative function	CG5935: function unknown but putative DNA-binding protein predicted to be involved in chromosomal organisation. The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can		
30		mRNA CG8648: Novel XPG/ flap endonu	clease-like, DNA repair protein	

Confirmation by RNAi Both show slight reduction of G1 peak

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Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

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CLAIMS

- 1. A polynucleotide selected from:
 - (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 1 to 70 or the complement thereof.
- 5 (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 1 to 70, or a fragment thereof.
 - (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 1 to 70 or a fragment thereof.
- 10 (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
 - 2. A polynucleotide selected from:
 - (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 1 to 14 or the complement thereof.
- 15 (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 1 to 14, or a fragment thereof.
 - (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 1 to 14 or a fragment thereof.
- 20 (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
 - 3. A polynucleotide selected from:

(a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 15 to 19 or the complement thereof.

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- (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 15 to 19, or a fragment thereof.
- 5 (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 15 to 19 or a fragment thereof.
 - (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

10 4. A polynucleotide selected from:

- (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 20 to 30 or the complement thereof.
- (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 20 to 30, or a fragment thereof.
- 15 (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in Examples 20 to 30 or a fragment thereof.
 - (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).

20 5. A polynucleotide selected from:

- (a) polynucleotides comprising any one of the nucleotide sequences set out in Examples 31 to 53 or the complement thereof.
- (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in Examples 31 to 53, or a fragment thereof.

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(c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in 31 to 53 or a fragment thereof.

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- (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
- 6. A polynucleotide selected from:
 - (a) polynucleotides comprising any one of the nucleotide sequences set out in 54 to 70 or the complement thereof.
 - (b) polynucleotides comprising a nucleotide sequence capable of hybridising to the nucleotide sequences set out in 54 to 70, or a fragment thereof.
 - (c) polynucleotides comprising a nucleotide sequence capable of hybridising to the complement of the nucleotide sequences set out in 54 to 70 or a fragment thereof.
- (d) polynucleotides comprising a polynucleotide sequence which is degenerate as a result of the genetic code to the polynucleotides defined in (a), (b) or (c).
 - 7. A polynucleotide probe which comprises a fragment of at least 15 nucleotides of a polynucleotide according to any of Claims 1 to 6.
- 8. A polypeptide which comprises any one of the amino acid sequences set out in Examples 1 to 70 or in any of Examples 1 to 14, Examples 15 to 19, Examples 20 to 30, Examples 31 to 53 and Examples 54 to 70, or a homologue, variant, derivative or fragment thereof.
- 9. A polynucleotide encoding a polypeptide according to Claim 8.
- 10. A vector comprising a polynucleotide according to any of Claims 1 to 7 and 9.

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- 11. An expression vector comprising a polynucleotide according to any of Claims 1 to 7 and 9 operably linked to a regulatory sequence capable of directing expression of said polynucleotide in a host cell.
- 12. An antibody capable of binding a polypeptide according to Claim 8.
- 5 13. A method for detecting the presence or absence of a polynucleotide according to any of Claims 1 to 7 and 9 in a biological sample which comprises:
 - (a) bringing the biological sample containing DNA or RNA into contact with a probe according to Claim 9 under hybridising conditions; and
- (b) detecting any duplex formed between the probe and nucleic acid in thesample.
 - 14. A method for detecting a polypeptide according to Claim 8 present in a biological sample which comprises:
 - (a) providing an antibody according to Claim 12;
 - (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and
 - (c) determining whether antibody-antigen complex comprising said antibody is formed.
 - 15. A polynucleotide according to according to any of Claims 1 to 7 and 9 for use in therapy.
- 20 16. A polypeptide according to Claim 8 for use in therapy.

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17. An antibody according to Claim 12 for use in therapy.

- 18. A method of treating a tumour or a patient suffering from a proliferative disease comprising administering to a patient in need of treatment an effective amount of a polynucleotide according to any of Claims 1 to 7 and 9.
- 19. A method of treating a tumour or a patient suffering from a proliferative disease,
 5 comprising administering to a patient in need of treatment an effective amount of a polypeptide according to Claim 8.
 - 20. A method of treating a tumour or a patient suffering from a proliferative disease, comprising administering to a patient in need of treatment an effective amount of an antibody according to Claim 12 to a patient.
- 10 21. Use of a polypeptide according to Claim 8 in a method of identifying a substance capable of affecting the function of the corresponding gene.
 - 22. Use of a polypeptide according to Claim 8 in an assay for identifying a substance capable of inhibiting the cell division cycle.
- 23. Use as claimed in Claim 22, in which the substance is capable of inhibiting mitosis and/or meiosis.
 - 24. A method for identifying a substance capable of binding to a polypeptide according to Claim 8, which method comprises incubating the polypeptide with a candidate substance under suitable conditions and determining whether the substance binds to the polypeptide.
- 25. A method for identifying a substance capable of modulating the function of a polypeptide according to Claim 8 or a polypeptide encoded by a polynucleotide according to any of Claims 1 to 7 and 9, the method comprising the steps of: incubating the polypeptide with a candidate substance and determining whether activity of the polypeptide is thereby modulated.

- 26. A substance identified by a method or assay according to any of Claims 21 to 25.
- 27. Use of a substance according to Claim 26 in a method of inhibiting the function of a polypeptide.
- 28. Use of a substance according to Claim 26 in a method of regulating a cell division5 cycle function.